2011

Central versus peripheral vascular mechanics: Early detection of vascular disease in young women who smoke

Chantelle Andrea Nielson

Central versus peripheral vascular mechanics:

Early detection of vascular disease in young women who smoke

(Spine Title: Early detection of vascular disease in young women who smoke)

(Thesis Format: Monograph)

By

Chantelle Andrea Nielson

Graduate Program

In

Kinesiology

Submitted in partial fulfillment of the requirements for the degree of

Master of Science

School of Graduate and Postdoctoral Studies

The University of Western Ontario

London, Ontario

THE UNIVERSITY OF WESTERN ONTARIO
SCHOOL OF GRADUATE AND POSTDOCTORAL STUDIES

CERTIFICATE OF EXAMINATION

Supervisor	Examiners
Dr. Kevin Shoemaker	Dr. Jim Dickey
Supervisory Committee	Dr. Alan Salmoni
Dr. Mair Zamir	Dr. Deborah O'Leary
Dr. Harry Prapavessis	

The thesis by

Chantelle Andrea Nielson

entitled:

Central versus peripheral vascular mechanics: Early detection of vascular disease

in young women who smoke

is accepted in partial fulfillment of the
requirements for the degree of
Master of Science

Date_____ _____
Chair of the Thesis Examination Board

ABSTRACT

This study tested the hypotheses that peripheral forearm but not central vascular mechanics are modified in young smokers but can be restored following a 14 week of intervention program of aerobic exercise and smoking cessation. Central and Peripheral vascular mechanics were compared in smokers pre (n=53) and post intervention (n=22) and age-matched non-smokers (n=25) (18-40 yrs). Measures included carotid to finger and toe pulse wave velocity, carotid and brachial arteries, and forearm vascular bed mechanics, to segment central and peripheral regions. Central vascular mechanics were not different between non-smokers and smokers. Compared to non-smokers, R, K, and L were greater and C was decreased in smokers ($p<0.05$). Relative to smoking baseline, R and L were reduced, C was increased ($p<0.05$), and K was unchanged following the intervention. In young smokers, in the absence of central changes smoking induced alterations in the periphery were reversible following an intervention program.

Keywords: Pulse wave velocity, Arterial Stiffness, Peripheral Vascular Mechanics, Cigarette Smoking, Vascular Disease

CO-AUTHORSHIP STATEMENTS

Maria F. Frances: Ms. Frances assisted with data collection and performed ultrasound assessments on some subjects.

Dr. Lyndsay Fitzgeorge: Dr. Fitzgeorge performed the recruitment and scheduling of the Getting Physical on Cigarette participants. She also performed the carbon monoxide test and conducted the majority of baseline anthropometric measures.

Dr. Kevin Shoemaker: Dr. Shoemaker assisted in the design of this study, guided the data analysis, and supervised the writing of this document.

ACKNOWLEDGEMENTS

I am deeply grateful to all who have made this thesis possible. These past years would not have been the same nor would this project would be what it is without the support and encouragement of many people. I would like to take this opportunity to offer a big, heartfelt thanks to those who contributed to this project.

To Dr. Kevin Shoemaker. Thank you so much for granting me all the amazing opportunities, learning experiences, and support as a member of the Neurovascular Research lab. I couldn't have asked for anything more during my Masters. Your enthusiasm and passion for science and research is truly contagious. You have encouraged me greatly in this field and allowed me to grow as both a researcher and person. Thank you for your constant support, encouragement, and advisory throughout this program. I cannot thank you enough.

Thank you to Dr. Harry Prapavessis and Dr. Mair Zamir for guiding this project development and providing valuable feedback as members of my advisory committee. Thank you for taking the time to meet with me individually to discuss data and results.

I would also like to thank my examining committee of Dr. Jim Dickey, Dr. Alan Salmoni, and Dr. Deborah O'Leary. In addition, thank you to Dr. Jim Dickey and Dr. Alan Salmoni for your support and guidance outside of this project.

Thank you to Dr. Lyndsay Fitzgeorge and the Exercise Psychology Lab. Not only were you essential to this project, the recruitment, organization, and ensuring it ran smoothly, but your constant support is extensively appreciated. Thank you for everything. And to all the volunteers, I thank you for giving up your time to assist in this project.

Thank you Arlene Fleischhauer who keeps us all on track and organizing the many schedules of the LBHH and NVRL.

Thank you to Maria Frances who helped me every step of the way. From teaching me how to conduct studies, learning ultrasound, proper data collection, and helping me test the majority of these subjects, thank you for all your time. Your contribution to this project has been enormous. And in addition to all of the above, thank you for your friendship, guidance, and for the wonderful experiences we have shared together. I will truly cherish those memories.

A big thank you for the constant support, discussions ('science talks'), and especially friendship from my wonderful labmates, Charlotte, Torri, Ruma, Katelyn, Nicole, Jasna, Aryan, Louis, Dylan, Janet, and Tamara. At the drop of a hat, anyone of you would take the time to help with posters, abstracts, presentations, and data, and I thank you for all of your help. Your contributions are immeasurable and your encouragement and assistance helped keep me on track. I cannot thank you enough for the friendships I have also gained and will treasure from the past 2 years.

I would also like to thank those individuals who have supported me outside the laboratory. To my friends, thank you for your constant support, encouragement, and excitement for me during this journey. Finally, to Mom, Dad, and Nathan. Thank you so much for supporting me every step of the way and giving me everything I could ever ask for. I could never have finished this project without your patience, love, and caring words of encouragement. Most of all, thank you for always believing in me.

And again, THANK YOU EVERYONE!!

Words cannot express how much gratitude I feel towards you all!

TABLE OF CONTENTS

CERTIFICATE OF EXAMINATION ..... **II**

ABSTRACT ..... **III**

CO-AUTHORSHIP STATEMENTS ..... **IV**

ACKNOWLEDGEMENTS ..... **V**

TABLE OF CONTENTS ..... **VII**

LIST OF FIGURES ..... **XI**

LIST OF TABLES ..... **XIII**

LIST OF ABBREVIATIONS ..... **XIV**

CHAPTER 1 : INTRODUCTION **1**

1.1 Background **1**

1.2 Purpose **4**

1.3 Hypothesis **4**

CHAPTER 2 : LITERATURE REVIEW **5**

2.1 Introduction **5**

2.2 Arterial Tree **5**

2.2.1 Tunica Intima 7

2.2.2 Tunica Media 8

2.2.3 Tunica Adventia 10

2.3 Components of the Arterial Tree to Regulate Blood Pressure and Flow **11**

2.3.1 Passive Properties 14

2.3.2 Active Properties 16

2.4 Properties of Steady State and Pulsatile flow in Central and Peripheral Vascular Mechanics **20**

2.5 Measuring Parameters and Changes in Central and Peripheral Vascular Mechanics **24**

2.5.1 Methods for Systemic Vascular Compliance 25

2.5.2 Local Vascular Compliance 28

2.5.3 Lumped Models to Study the Entire Vascular Bed 29

2.5.4 RCKL Model 30

2.6 Interim Summary **33**

2.7 Vascular Disease **34**

2.7.1 Risk Factors for Vascular Disease 34

2.8 Smoking and Vascular Disease **35**

2.8.1 Progression of Vascular Disease 36

2.8.2 Changes in Vascular Mechanics in Smokers and Vascular Disease 37

2.8.3 Mechanisms of Vascular Disease Progression in Smokers 39

2.9 Reversing Altered Vascular Mechanics **43**

2.9.1 Exercise Training 44

2.9.2 Smoking Cessation 45

2.10 Purpose **46**

2.11 Hypothesis **46**

CHAPTER 3 : TOOLS AND TECHNIQUES **47**

3.1 Brachial Artery Pressure **47**

3.2 Ultrasound **49**

3.2.1 Ultrasonic Imaging 50

3.2.2 Doppler Ultrasound 52

3.2.3 Validity 54

CHAPTER 4 : METHODS **56**

4.1 Subjects **56**

4.2 Recruitment **56**

4.3 Protocol Design **57**

4.4 Lifestyle Intervention – Aerobic Exercise and Smoking Cessation **58**

4.5 Data analysis **59**
4.5.1 Vascular Mechanics 59
4.5.2 Arm Volume 62

4.6 Statistical Analysis **62**

CHAPTER 5 : RESULTS **64**

5.1 Systemic Haemodynamics **64**

5.2 Central Vascular Mechanics **64**
5.2.1 Pulse Wave Velocity 64
5.2.2 Carotid Artery Characteristics 65
5.2.3 Local Carotid Mechanics 65

5.3 Peripheral Vascular Mechanics **65**
5.3.1 Brachial Artery Characteristics 65
5.3.2 Local Brachial Mechanics 65
5.3.3 Peripheral Forearm vascular properties 66

5.4 Subject Characteristics **69**

5.5 Systemic Haemodynamics **69**

5.6 Central Vascular Mechanics **70**
5.6.1 Systemic Vascular Stiffness -Pulse Wave Velocity 70
5.6.2 Carotid Artery Characteristics 70
5.6.3 Local Carotid Mechanics 70

5.7 Peripheral Vascular Mechanics **70**
5.7.1 Brachial Artery Characteristics 70
5.7.2 Local Brachial Mechanics 71
5.7.3 Peripheral Forearm vascular properties 71

5.8 Sub Analysis **78**
5.8.1 Internal Controls 78
5.8.2 Regression/ANCOVA Analysis 78

CHAPTER 6 : DISCUSSION **83**

6.1 Hemodynamic Variables **83**

6.2 Central Vascular Mechanics **84**

6.3 Peripheral Vascular Mechanics **86**

6.4 Effect of a Lifestyle Intervention on Peripheral Vascular Mechanics **89**

6.5 Correlation Analysis **93**

6.6 Implications **94**

6.7 Limitations **94**

CHAPTER 7 : CONCLUSION **96**

APPENDIX 1 : USE OF HUMAN ETHICAL APPROVAL NOTICE **120**

CURRICULUM VITAE **122**

LIST OF FIGURES

Figure 2.1 - Forward and reflected waveforms and the effect on blood pressure.....14

Figure 2.2 - Variety of compositional components of the blood vessles.....15

Figure 2.3 Neural, mechanical, and chemical factors that regulate blood flow.....20

Figure 2.4 Resistance: Mean Arterial Pressure and Mean Blood Flow. 22

Figure 2.5 Measurement of Pulse Wave Velocity from a sample set of data. 26

Figure 2.6 RCKL Model. 32

Figure 2.7 Likelihood of carotid atherosclerosis as a function of age and smoking.....36

Figure 4.1 Carotid Artery Ultrasound Image 61

Figure 5.1 - Forearm vascular mean arterial pressure, flow, and resistance. 67

Figure 5.2 Forearm vascular compliance, viscoelasticity, and intertia. 68

Figure 5.3 Forearm vascular resistance following the intervention program. 72

Figure 5.4 Forearm mean arterial pressure following the intervention program. 73

Figure 5.5 Forearm blood flow following the intervention program. 74

Figure 5.6 Forearm vascular compliance following the intervention program.....75

Figure 5.7 Forearm vascular viscoelasticity following the intervention program 76

Figure 5.8 Forearm vascular inertia following the intervention program.....77

Figure 5.9 Regression Analysis of Smoking Quantity vs. Central Mechanics 79

Figure 5.10 Regression Analysis of Smoking Quantity vs. Central Mechanics (2). 80

Figure 5.11 Regression Analysis of Smoking Quantity vs. Peripheral Local Mechanics.81

Figure 5.12 Regression Analysis of Smoking Quantity vs. Peripheral Mechanics. Determined from the RCKL Model.. 82

LIST OF TABLES

Table 2.1 Local Arterial Stiffness Equations 29

Table 5.1 Subject Characteristics 64

Table 5.2 Local Carotid Mechanics 65

Table 5.3 Local Brachial Characteristics 66

Table 5.4 Smoking Cessation Subject Characteristics 69

Table 5.5 Local Carotid Mechanics for individuals before (Pre) and following (post) a 14 week Lifestyle Intervention Program 70

Table 5.6 Local Brachial Characteristics for individuals before (Pre) and following (post) a 14 week Lifestyle Intervention Program 71

Table 5.7 Forearm RCKL Parameters for individuals who did not quit smoking at baseline (Smokers) and at follow-up (14 weeks) 78

LIST OF ABBREVIATIONS

BP	Blood Pressure (mmHg)
C	Compliance (mL/min) (determined from RCKL model)
DBP	Diastolic Blood Pressure (mmHg)
EDRF	Endothelium derived relaxation factor
HR	Heart Rate (beats/min)
IMT	Intima-Media Thickness
K	Viscoelasticity (mmHg/mL/min) (determined from RCKL model)
L	Inertia (mmHg/mL/min^2) (determined from RCKL model)
MAP	Mean Arterial Pressure (mmHg)
PP	Pulse Pressure (mmHg)
PWV	Pulse Wave Velocity (cm/s)
Q	Cardiac Output (L/min)
R	Resistance (mmHg/mL/min) (determined from RCKL model)
RTF	Return-to-flow
SBP	Systolic Blood Pressure (mmHg)
SV	Stroke Volume (mL)
TPR	Total Peripheral Resistance (mmHg/L/min)

CHAPTER 1 : INTRODUCTION

1.1 Background

Vascular disease, a disease affecting the arterial vasculature, is a leading cause of death among Canadians (153). Early detection of vascular disease would significantly impact intervention strategies and the promotion of healthy living. Although many clinical and research risk factors have been heavily investigated and identified, even earlier detection may improve intervention programs and the understanding of the progression of this disease.

The clinical determination of vascular disease relies heavily on measures of central artery vascular mechanics. Common detection analyses study the large conduit vessels and quantify markers of disease such as intima media thickness (IMT), local compliance and distensibility, pulse pressure (PP), augmentation index, and pulse wave velocity (PWV). Although these measurements are correlated to the likelihood of developing vascular disease, a major limitation of these parameters is that they investigate mechanics of large, central arteries only. Recent, evidence suggests that downstream changes in the small, peripheral resistance arterioles may precede these central changes (93). Therefore, simply investigating markers of central vascular stiffness does not adequately describe the arterial system (210).

The vascular properties that regulate blood pressure (BP) and blood flow are important to study as in disease states, these parameters are altered and may provide evidence for the progression of vascular disease. These BP and flow waveforms travel throughout the arterial vasculature in the large, central conduit arteries through the many branches to the small, peripheral resistance arterioles (137). The central and peripheral

arteries of the cardiovascular system play an important role in the conduction of blood flow to the periphery. The elastic properties of these vessels allow for the smoothing of oscillations in BP, reduction of the PP, and perfusion of the organs. These properties are dependent on the composition and geometry of the walls of the arteries, which determine the velocity of the propagation of the forward and backward (reflected) travelling pressure waves and resulting blood flow. Forward pressure waves generated from the heart can be reflected at branching points, areas of alteration in arterial geometry, or terminal arterioles and this reflected pressure waveform can alter blood flow in the central arteries. Kember and colleagues (2004) demonstrated that this dynamic pattern of central PP is determined by downstream conditions, or information from the periphery, as global information is required for adequate cardiovascular control (93). In a stiffer, peripheral vascular bed, the reflected pressure waveform will travel faster backup towards the heart. This increased velocity of the reflected waveform will reach the heart earlier in systole, causing a higher systolic pressure. In cases of aging or diseased vessels, the constant attenuation in BP may lead to vascular remodelling. Thus, through pressure distribution, the affect of peripheral alterations on the control of BP regulation can affect central arteries (93; 109). Therefore, the properties regulating BP and blood flow in peripheral arteries may serve as an early marker of disease.

The properties regulating BP and delivery of blood flow are determined by many parameters throughout the vasculature that contribute to the steady, continuous flow, and the pulsatile, oscillatory dynamics of blood flow (178). Resistance is the pressure that the heart must overcome to deliver adequate blood to the periphery. Resistance equals mean arterial pressure (MAP) divided by cardiac output (Q), the amount of blood ejected per

minute from the heart (137). While it was previously believed that resistance was the only parameter regulating blood flow, this component only deals with the steady state. Blood flow is pulsatile and, therefore, during each cardiac cycle, other parameters of the vessel wall regulate both the pressure and flow waveforms (210). These other parameters have been identified as the compliance or elasticity of vessel wall, the viscoelasticity, or resistance to stretch of the vessel wall, and the inertia of the blood and tissues (210; 211). While the steady state components of blood flow have been studied extensively, the pulsatile components are not as well known. Additionally, these parameters vary throughout the vascular tree and these properties in the periphery are not well studied. Therefore central and peripheral dynamic vascular properties should be investigated to understand the entire arterial tree and vascular mechanics.

The inadequate understanding of peripheral vascular mechanics is due to the limitations of studying these small vessels in vivo. Recently our lab has developed a model to study the peripheral vascular bed using a mathematical Windkessel model (210; 211). This model incorporates the resistance (R), compliance (C), viscoelasticity (K), and inertia (L) of the downstream vasculature. These parameters are regulated by both the passive and active properties of the blood vessel. The ability to detect changes in these parameters, namely R, C, K, and L in the peripheral vascular bed may help improve detection of vascular disease.

In this study, smoking was used as a model for vascular disease. Smoking, the number one preventable risk factor for vascular disease (188), is an ideal model to study the central and peripheral alterations that may occur in a disease state. While smoking has been shown to have profound effects on central, steady state, vascular mechanics, the

parameters affecting blood flow in the periphery remained to be investigated. Young individuals, without vascular disease, represent a sample population to study to investigate these early changes, without the confounding factors of aging. Separating the arterial tree into central and peripheral vascular mechanics may allude to vascular alterations and progression in disease states.

1.2 Purpose

The purpose of this study was to investigate central and peripheral arterial vascular mechanics in young smokers and non-smokers. The second purpose was to determine whether these altered vascular mechanics can be improved following a 14 week intervention program of aerobic exercise and smoking cessation program.

1.3 Hypothesis

It was hypothesized that vascular mechanics will be affected detrimentally in the peripheral vascular bed of young smokers in the absence of central changes. This hypothesis predicts that, compared to non-smoking control subjects, peripheral vascular mechanics will be altered in young smokers. Despite these changes in the periphery, it is hypothesized that central vascular measurements will not be different between non-smokers and smokers. The second hypothesis was that these altered peripheral vascular mechanics can be restored in young smokers following the 14 week combined intervention program.

CHAPTER 2 : LITERATURE REVIEW

2.1 Introduction

To fully understand vascular mechanics and vascular disease, one must investigate the arterial vasculature. Therefore this literature review examines: 1) vascular tree anatomy, 2) the components regulating BP and flow, 3) central and peripheral vascular mechanics, 4) how we can measure and quantify central and peripheral vascular mechanics, 5) how vascular disease may alter these components, 6) how to quantify the impact of smoking on the segmental vascular tree mechanics, and 7) whether such changes can be reversed through lifestyle intervention.

2.2 Arterial Tree

The cardiovascular system in a human is designed to transport blood throughout the body, delivering oxygen and nutrients essential for living (137; 168). The circulation of blood consists of two circuits, the pulmonary and the systemic circuit (137). For this review, we will focus on the systemic circulation, and more specifically, the arterial side as it pertains to vascular mechanics.

The systemic circulation begins with the heart, which acts like a pump to distribute blood to all parts of the body. The circulation of blood flow from this pump was recognized by William Harvey in 1628 and was simplified into a model of blood leaving the left ventricle and returning to the right atrium (137; 163). This single loop model does not identify the arterial branching network that encompasses the systemic circulation and the physiological control of the circulation (12). The vascular system has the hierarchical form of a tree structure, as the arteries branch from the major root, the aorta, and travel along a branching system to terminal arterioles and thus the capillaries. It is in the

capillaries where vital nutrients and oxygen can be transmitted to organs and muscle (137). The capillaries also extract waste and veins carry the oxygen- depleted blood back to the heart and pulmonary circulation (137).

Once blood leaves the heart, the arteries receive the blood at a high pressure and velocity and conduct it throughout the body (137). Pressure waveforms are transmitted from the heart and reflected at terminal arterioles and at various obstacles, such as bifurcations, and, therefore, the arrangement of this branching network affects the propagation and reflection of waveforms (179). The branching networks of arteries function to cushion the high pressure generated from the heart and transform this flow to the periphery (140; 179). The first branches from the heart are large, conduit, elastic arteries containing substantial amounts of elastin that allow the vessels to inflate and recoil while transmitting pulsatile flow generated from the heart. These large vessels are not only elastic, but also contain large amounts of fibrous tissue to provide strength to cushion pressure (11; 16). The periphery, or small arteries and arterioles, act as control valves (16; 137). These arteries are often referred to as muscular arteries, as they are less compliant, but capable of active changes in vessel diameter (16).

The transition from arteries to arterioles is a gradual one and is associated with a progressive reduction in the calibre of the lumen and thickness of the vessel wall (168). The design of the vascular wall is also altered to account for the vessel's function in regulating blood flow. The geometry and composition of the large (central) versus smaller (peripheral) arteries will be discussed further in Section 2.3. Although the components of the wall vary between large and small arteries, they all consist of three vital layers with

different functions: 1) the tunica intima, the innermost layer; 2) the tunica media, the middle layer; 3) the tunica adventitia, the outermost layer.

2.2.1 Tunica Intima

The tunica intima, is the inner surface of the arterial vessel. The endothelium is the predominant component of the tunica intima. This layer is in contact with the blood and therefore interacts with the components of the blood and responds to the velocity and volume of blood flow (137; 168). The endothelium is uniquely positioned between the vascular smooth muscle and the blood and performs multiple functions in the physiology of circulation (140). It was previously believed that the only function of the endothelium was to act as a barrier between the blood and vessel, but the Nobel prize winning observation that acetylcholine-mediated relaxations were dependent on an intact endothelium provided evidence as to the considerable biological potential of the endothelium (60). Endothelium-derived relaxation factors (EDRFs) are released to cause vasorelaxation, and perform the pivotal role of the endothelium (196). The existence of an EDRF was postulated by Furchgott and Zawadski (1980) when the relaxant action of acetylcholine in pre-constricted rabbit aorta strips was abolished in strips with the endothelium removed. This loss of relaxation was quantitatively related to the histological measured loss of endothelial cells. Subsequently, it was discovered that one of the major EDRFs is nitric oxide, a biological agent that helps maintain arterial tone in the vasculature (79). Nitric oxide, which is mainly synthesized in the endothelial cells, is a signalling molecule that is produced from activation of the endothelium (140). Prostacyclin has been identified as another EDRF; however, its role in endothelial function and disease is not as well known as nitric oxide. The relaxation through cellular signalling increases the diameter of blood vessels, thus increasing flow and decreasing

pressure. The endothelial layer also performs other distinctive functions: it is involved in the regulation of coagulation, leukocyte adhesion in inflammation, vessel relaxation and constriction, and vascular smooth muscle growth (140).

Nitric oxide release results in vascular relaxation and inhibits leukocyte adhesion to the endothelium (56). Nitric oxide bioavailability is therefore essential for the maintenance of endothelial function and vasorelaxation. Various studies illustrate that alterations in the release of vasodilators synthesized by the vessel wall occur at an early stage in the atherosclerotic process (77; 99; 121; 134; 136). The vascular endothelium regulates arterial relaxation and contraction, controls platelet and leukocyte interactions, and controls vascular growth (137; 140; 169).

2.2.2 Tunica Media

The second or middle layer, is known as the tunica media and consists of smooth muscle cells and elastic fibres (168). In larger vessels, elastin fibres dominate the media layer while as the arteries become smaller, the number of smooth muscle fibres increase (179). Unlike endothelial cells which are arranged longitudinally along the vessel, smooth muscle cells are found in a circular pattern around the blood vessel, though some are oriented in parallel (50; 137; 168). The tight helical or circular arrangement of smooth muscle cells allow for control of vessel diameter by vasoconstriction or vasodilation (168). The diameter of the vessel controls the resistance, as a change in diameter will alter resistance and thus blood flow. Poiseuille's Law states that resistance is inversely related to the radius to the fourth power (137). Therefore a small change in diameter will have a large impact on resistance.

Morphological studies have compared the contractile components of smooth muscle cells to that of striated muscles (179). The contractile components of smooth muscle cells consist of actin and myosin and are regulated by intracellular calcium levels. Unlike the endothelial cell, which produces dilatory compounds in response to increased calcium levels, smooth muscle cells constrict with increasing calcium (167; 172). Two mechanisms can contribute to increased calcium concentrations inside smooth muscle cells. 1) Depolarization of the smooth muscle is followed by the opening of L-type voltage gated channels. This influx leads to further release of calcium from intracellular stores; and 2) Binding of a hormone to its receptor leads to an increase in intracellular calcium via receptor-operated calcium channels or G-protein coupled activation. Increased calcium inside the cells allows actin-myosin interaction and the shortening of smooth muscle cells (27; 167).

Previously it was thought that smooth muscle contraction or dilation affected the diameter, or resistance of the vessel only. This is incomplete due to the pulsatile nature of blood flow as smooth muscle responses also affect arterial compliance or stiffness. Smooth muscle relaxation has been shown to alter the wall stiffness or elastic modulus (15). Smooth muscle can increase stiffness by contracting the collagen and elastin fibres in parallel with the smooth muscle (33; 165). However, it also decreases stiffness by reducing the tension in the smooth muscle itself and in the series elastic component. Therefore the net effect depends on the contribution of each of these components in the vessel wall.

Smooth muscle cells found in the tunica media provide a mechanism to regulate arterial mechanical properties (179). Smooth muscle cells are highly innervated by the

sympathetic nervous system, interact with the endothelium, and exhibit receptors for various regulatory molecules (53). A wide variety of stimuli activate the contractile properties of smooth muscle cells.

2.2.3 Tunica Adventia

The outermost layer, the tunica adventia, is composed of collagen and elastin fibres (168). Collagen is a connective tissue protein that gives the vessel strength and protects the arteries from the high pressure, pulsatile flow generated from the heart. The elastin enables the vessel to recoil to contribute to the propagation of pressure and flow throughout the circulation (37). The ratio of collagen to elastin is thought to be a major contributor to the compliance or distensibility of a vessel (55; 57).

Elastin fibres are found in both the tunica media and adventia layers. Elastin is a protein in connective tissue that coils and recoils similar to a spring and accounts for the elasticity of the vessel wall at low pressures (57). Elastin is important in arteries as it enables distensibility that, in turn, functions to propagate pressure waves and transmit blood flow throughout the systemic circulation. The basic structure of elastin enables arteries to function as a mechanism to buffer and store pulsatile flow as it is able to distribute forces throughout the wall (179). Within each cardiac cycle, elastin allows the vessel to expand to buffer the pressure increase, store the increased volume of flow, and transmit this volume to the various parts of the body.

Vessel walls are not purely elastic because collagen, a connective protein fibre, provides the tensile strength at higher pressures. Collagen is predominately found in the tunica adventia (168; 179). The collagen fibres are flexible, yet they have considerable strength. The fibres are usually arranged in parallel bundles and are recruited as

transmural pressure increases to bear mechanical load (16). Collagen spreads the pulsatile load of the pulse wave and thus prevents overstretching of the elastic element (41). The structure of collagen is fundamentally different from that of elastin as it is composed of closely-wound helical chains which are tightly cross-linked, thereby restricting their distensibility. Comparing the elastic modulus, an engineering measure of stiffness, of collagen and elastin indicates that collagen is several hundred to one thousand times as stiff as stretched elastin (49).

Each branch and change in vessel wall composition is important in the haemodynamics of blood flow. Pressure waves transmitted from the heart are altered with changes in vessel wall properties and reflected at bifurcations and terminal arterioles, which can be regarded as an obstacle to flow. Since William Harvey, the precise information on the effect of the different components, size, branches, and physiological control of the circulation have been investigated to determine the effect BP and flow have on the design on the arterial vessels. Throughout the arterial vasculature, the cellular make-up and regulatory mechanisms are appropriate for the pressure and flow that the segment is subjected to. Therefore, it is important to study the segments of the arterial vasculature separately to understand the parameters regulating pressure and flow in each area. The changes through aging and disease have yet to be fully elucidated.

2.3 Components of the Arterial Tree to Regulate Blood Pressure and Flow

The concept of haemodynamics is concerned with the relationships governing pressure, flow, and volume within the cardiovascular system. The amount of blood flow that is delivered to various parts of the body is controlled by many different mechanisms. Most importantly, blood flow follows BP and the mechanisms regulating BP are valuable

to understand blood flow. Arterial PP is an important signalling hemodynamic parameter regulating blood flow.

BP is comprised of two components: steady state and pulsatile (109; 210). The steady-state component is characterized by the MAP, which represents the product of Q and peripheral resistance (161). The pulsatile component is determined by the left ventricular ejection and stroke volume (SV), as well as the compliance, or elasticity and recoil, of the arterial circulation (120). The elastic wall of the aorta transforms the pulsatile on-off blood flow of the left ventricle into the less pulsatile flow of the distal arteries (137). During each cardiac cycle, arteries exhibit oscillations in diameter as arterial pressure causes arteries to distend circumferentially.

Kember and colleagues (2004) demonstrated that this dynamic pattern of PP is determined by downstream conditions, or information from the periphery, as global information is required for adequate cardiovascular control (93). This study demonstrated that peripheral vascular mechanics can alter blood flow in the central aorta. The periphery controls the speed of the reflected wave that will merge with the forward wave in the aorta, thus affecting BP and flow in the central arteries.

Forward pressure waves generated from the heart can be reflected at branching points, areas of alteration in arterial geometry, or terminal arterioles, and parts of the wave are reflected upstream to alter blood flow in the aorta. Reflected waves travel backwards with approximately the same speed as the forward-flowing wave and, at some point through the aorta and its branches, the incident and reflected waves are traditionally thought to

summate. The wave speed of the forward and reflected wave depends on the diameters, lengths, and elasticity of the vessels (137; 140; 179).

Pressure wave reflection may serve at least two beneficial purposes (109). First, the reflected wave returns to the central aorta in diastole, thereby enhancing the diastolic perfusion pressure in the coronary arteries (137). Secondly, wave reflection limits the transmission of pulsatile energy to the periphery where damage to the microcirculatory beds might occur (162).

However, in a stiffer system, the wave speed of the reflected wave is increased. This increased wave speed may diminish wave reflections or cause wave reflections to return earlier, and may lead to augmentation of systolic pressure due to the fusion of the forward and reflected waves earlier in the cardiac cycle (137; 202). A stiffer vascular bed produces less buffering of systolic blood pressure (SBP) and less recoil in diastole resulting a greater fall in diastolic blood pressure (DBP). This increased systolic pressure and reduced diastolic perfusion may limit steady state flow in the coronary circulation and increase transmission of pulsatile energy into the microcirculation (162).

Figure 2.1 demonstrates how the downstream vasculature can affect the dynamic PP in the upstream central vasculature. Specifically, a stiffer vascular bed will cause faster reflected pressure waveforms that backup towards the heart. If they travel faster, as in a stiffer vessel, they will reach the heart earlier in systole, causing a higher systolic pressure. In cases of aging or diseased vessels, the constant augmentation in BP may lead to vascular remodelling. Thus, through pressure distribution, the effect of peripheral alterations on the control of BP regulation can affect central arteries (93; 109).

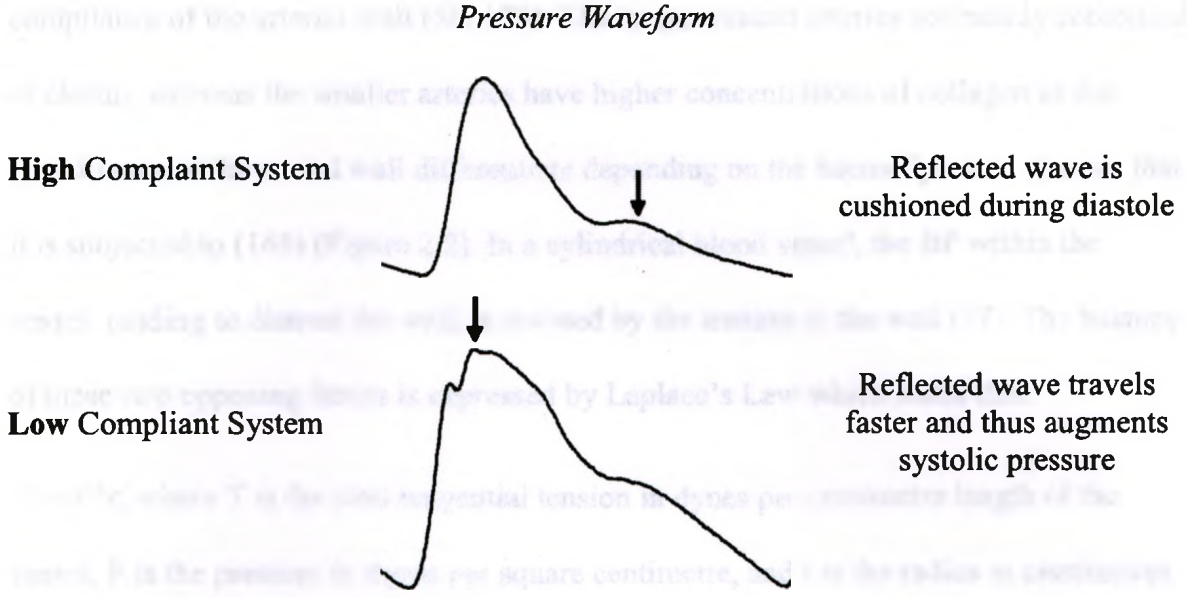


Figure 2.1 - Forward and reflected waves are determined by the stiffness or compliance of the arterial walls. The recorded pressure waveform is the summation of the forward and reflected waveforms. The downward arrows indicate the location of the reflected wave.

The peripheral and central vascular mechanics and regulation of BP are controlled by both passive and active properties. Therefore the mechanical, anatomical, and physical properties of the vessel are important to understand the mechanics that regulate the blood vessels' ability to distribute changes in pressure.

2.3.1 Passive Properties

Elastic modulus, an engineering term to describe the deformation of an object when a force is applied to it, indicates the elasticity or ability of an object to return to its original shape and size (137). The elastic modulus is high for collagen and low for elastin. Since collagen is a stiff, high elastic modulus fibre whereas elastin is easily expanded and returns to its original form, it has been suggested that the relative proportion of elastin and collagen directly determines the overall elastic modulus of the different blood vessels (55). The ratio of collagen to elastin is suggested as a useful index to determine the

compliance of the arterial wall (50; 179). The large, conduit arteries are mainly composed of elastin, whereas the smaller arteries have higher concentrations of collagen as the constituents of the vessel wall differentiate depending on the haemodyanmic stresses that it is subjected to (168) (Figure 2.2). In a cylindrical blood vessel, the BP within the vessel, tending to distend the wall, is resisted by the tension in the wall (37). The balance of these two opposing forces is expressed by Laplace's Law which states that:

$T = P*r$; where T is the total tangential tension in dynes per centimetre length of the vessel, P is the pressure in dynes per square centimetre, and r is the radius in centimetres. It has been suggested that the absolute level of tension determines the collagen content in the vessel wall and that the rate of change of tissue tension determines the elastin content (137). The regulation of wall tension is a main determinant of travelling waves as both forward and reflected waves are determined by the elasticity of the vessel walls.

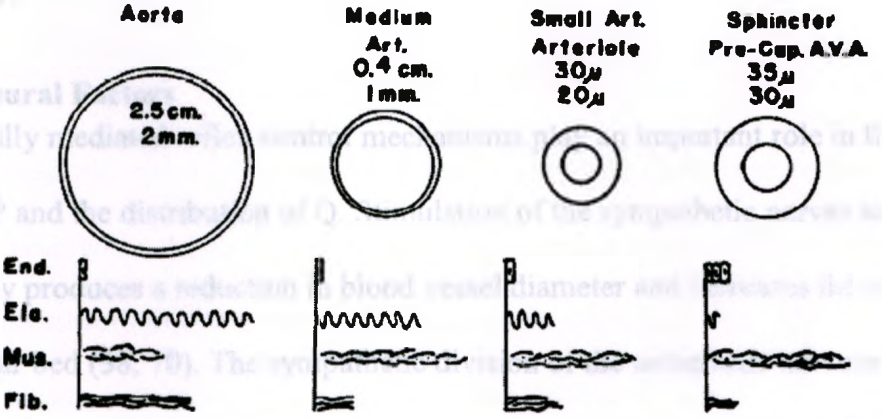


Figure 2.2 - Variety of compositional components of the four tissues of difference blood vessels. The first number under the artery name is the diameter of the lumen. The second number is the thickness of the wall (37).

While Laplace's Law identifies some factors that contribute to the mechanical properties of blood vessels, it does not account for other factors (in addition to simply the passive properties), or the total amount of connective tissue proteins present. Although studies have demonstrated a close agreement between the collagen-elastin ratio of blood vessels and mechanical properties of both normal and diseased vessels, this relationship does not hold for peripheral arteries (55; 179). Since large variations in composition occur in these arteries, it can be concluded that other factors, such as the active properties of the arterioles, contribute to the determination of the mechanical properties of blood vessels in addition to simply the total amount of collagen and elastin present (179).

2.3.2 Active Properties

Changes in the pressure and flow through the vasculature signal various reflex mechanisms that alter the structure, growth, and differentiation of the vessel wall. These active mechanisms of vascular control are regulated neurally, mechanically, and chemically.

2.3.2.1 Neural Factors

Neurally mediated reflex control mechanisms play an important role in the control of arterial BP and the distribution of Q. Stimulation of the sympathetic nerves to a vascular bed usually produces a reduction in blood vessel diameter and increases the resistance in the vascular bed (38; 70). The sympathetic division of the autonomic nervous system innervates the entire arterial tree down to the smallest arterioles (53; 161). The arterioles are highly innervated by the sympathetic nervous system and represent a level for greater control compared to the larger arteries (138). Therefore it is important to measure blood flow changes both centrally and in the periphery.

Though the sympathetic nervous system was previously believed to control the vasculature via changes in resistance only (38; 70), this concept deals primarily with a constant flow model of the circulation, whereas blood flow is pulsatile (210). In recent years, it has been increasingly recognized that the compliance of blood vessels may be an important property regulating the control of blood flow (105; 161; 183; 210). Supporting this notion, previous work on the neurovascular control of blood vessels has shown that the sympathetic nervous system can affect the compliance of a blood vessel without changes in vessel diameter (165; 211). A change in the compliance of a blood vessel will affect the rate of blood supply to a vascular bed (210) as a reduced compliance may decrease the transit time of blood flow through the circulation.

2.3.2.2 Mechanical Factors

An intrinsic property of the arterial vasculature, and more specifically smooth muscle cells, is the myogenic response which was identified by Bayliss in 1902 (22). If the smooth muscle cell is stretched, it responds by contracting. This feature provides automatic responsiveness to changes in mechanical load (137).

The myogenic response is capable of regulating blood flow since the vessel wall of regulatory vessels is predominantly composed of smooth muscle cells and therefore a change in pressure will affect the vessel diameter. In addition to the local changes, the myogenic response feeds back to the systemic BP and contributes to the protection of fluctuations of pressure (27; 50; 85). This is achieved by the change in wall tension, signalling membrane depolarization, and an influx of calcium via opening of voltage operated calcium channels. This promotes myosin actin interaction and vasoconstriction occurs (167). Although the myogenic response is thought of as a vasoconstrictive

response only, altering transmural pressure has been shown to change compliance without a change in resistance (58; 211).

2.3.2.3 Chemical Factors

Various substances, such as hormones, blood borne factors, and endothelial derived molecules are vasoactive and can attenuate or augment mechanical and neurogenic effects. These metabolites can have direct or indirect actions on the vessel wall.

Hormonal control of blood vessels plays an important role in the control of the cardiovascular system. There are a number of hormones that when released into the blood, produce significant effects on vascular diameter and elasticity. Catecholamines are released from the adrenal medulla, and circulate through the blood during sympathetic stimulation and have both direct and indirect effects on the vessel wall (161).

Sex hormones also affect vascular control and may account for some differential responses between men and women. Estrogen, a hormone present in both sexes but at a higher concentration in women, is a vasodilatory stimulus (76; 128). Testosterone, another hormone present in both sexes, can be converted to dihydrotestosterone by 5α-reductase or to estrgoen by aromatase (114). Circulating levels of testosterone are generally high in men and low in women and testosterone and its conversion to 17β-estradiol is important in both men and women as a key sex steroid (128). The conversion of testosterone to 17β-estradioal via aromatase, is an important source of estrogen, as it represents approximately 80% of available 17β-estradiol in men and may contribute to the regulation of vasodilator function in men (106; 128). The estrogen-induced vascular relaxation pathways involve changes in the synthesis, release, and thus bioavailability of

EDRFs such as nitric oxide, prostacyclin, and the endothelium-derived hyperpolarizing factor (52; 77).

In addition, estrogen supplementation has been reported to maintain or improve arterial compliance (43; 92; 155). Zhang *et al.* (2001) reported that endogenous estrogen mediates vascular reactivity and distensibility in pregnant rats (215) and estrogen replacement reduces age-associated remodelling in normal rats (214). It was further demonstrated that high expression of alpha estrogen receptors are correlated with a lower collagen concentration as well as the stiffness of human uterine arteries (111). This estrogen mediated protection against increased collagen may improve vessel distensibility and compliance. However, the effect of estrogen on vascular stiffness may not be homogeneous in different regions of the vascular bed as estrogen treatment increases aortic stiffness and potentiates endothelial vasodilator function in the hindlimb, but not in the carotid vascular bed of rats (186).Therefore, the concentration of estrogen in both males and females will affect vasodilatory function, vascular geometry, and vascular stiffness.

These components regulating vascular mechanics work independently and dependently with one another depending on the availability and interaction between these mechanisms. Understanding how these components regulate vascular mechanisms is important to study the changes that may occur in the control of the cardiovascular system (Figure 2.3). Alterations in both passive and active properties will thus affect BP and blood flow.

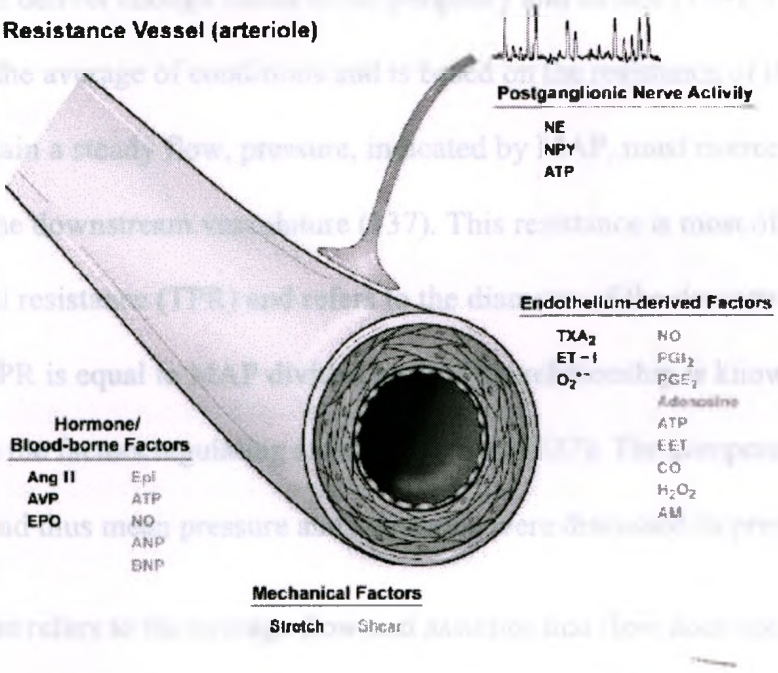


Figure 2.3 Summary of major neural, mechanical, and chemical factors that determine various properties regulating blood flow in the skeletal muscle circulation. Factors causing constriction are shown in black and dilatory factors are shown in gray. Ang II, angiotensin II; AVP, arginine vasopressin; Epi, epinephrine; ATP, adenosine triphosphate; ANP, atrial natriuretic peptide, BNP, brain natrieretic peptide; NE, norepinephrine, NPY, neuropeptide Y; TXA2, thromboxane; ET-I, endothelin-I; O2-, superoxide; NO, nitric Oxide; PGI2, prostacyclin; PGE2, prostaglandin E2; EET, eicosatrienoic acid; CO, carbon monoxide; H2O2, hydrogen peroxide; AM, adrenomedulin. (Adapted from reference 131)

2.4 Properties of Steady State and Pulsatile flow in Central and Peripheral Vascular Mechanics

The factors regulating vascular mechanics have been discussed briefly in the above sections and although the vessel wall components and neural, mechanical, and chemical inputs will alter vascular mechanics, simply studying these factors do not identify the parameters regulating blood flow to supply the living organism. A more integrated view of vascular mechanics considers both the steady component and the pulsatile component.

To ensure adequate delivery of blood flow for metabolic exchange, the conduit arteries need to deliver enough blood to the periphery and tissues (179). The steady component is the average of conditions and is based on the resistance of the vascular tree (12). To maintain a steady flow, pressure, indicated by MAP, must overcome the resistance of the downstream vasculature (137). This resistance is most often defined as total peripheral resistance (TPR) and refers to the diameter of the downstream vasculature. TPR is equal to MAP divided by Q. This relationship is known as Ohm's law and represents the factors regulating steady state flow (137). The components regulating the diameter and thus mean pressure and resistance were discussed in previous sections.

Steady state refers to the average flow and assumes that flow does not vary with time, which in fact is not true of arterial flow. It is generally taught that the control of peripheral blood flow is regulated by a change of peripheral resistance based on Poiseuille's law. These assumptions are not directly related to in vivo conditions as arterial flow is pulsatile and the diameter of the arterial vessel changes within each cardiac cycle (210). Although important, resistance cannot be the only parameter determining blood flow, because if this were the case, the flow waveform would be similar to that of pressure; but it is not (Figure 2.4). In as early as 1943, Shipley *et al.* studied pulsatile flow patterns in a wide range of arteries and found no clear relation between the pressure and flow waveforms (170). In all areas of the circulation it can be seen that this is not the case and other mechanisms regulating the dynamic or oscillatory components of blood flow must be important (210).

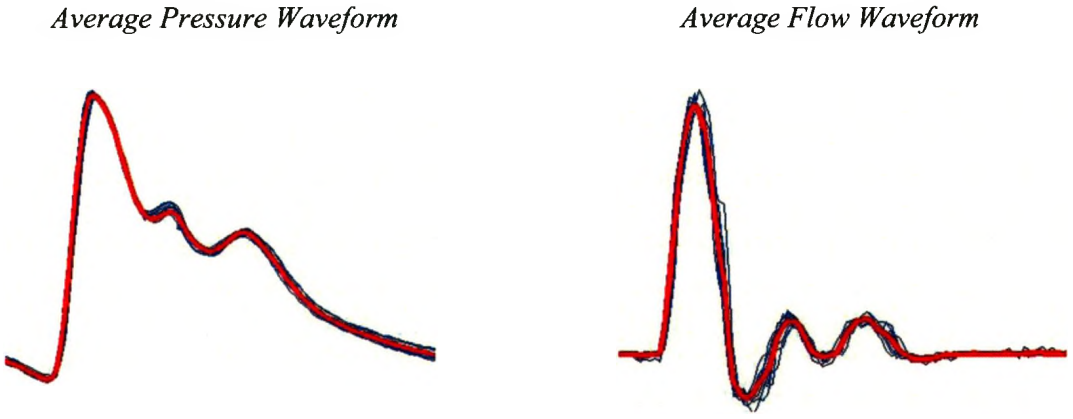


Figure 2.4 Pressure (Left) and Flow (Right) Waveforms. If resistance was the only parameter regulating blood flow, the shape of the waveforms would be similar. This is not the case and other parameters must contribute to blood flow.

The compliance, or elasticity, of the vessel wall is another parameter affecting pulsatile blood flow (210). Vascular compliance is determined by the change in volume (or diameter) of a blood vessel segment for a given pressure change (179). Vascular elasticity is determined by factors that regulate the diameter of a blood vessel and the elastic components in the vessel wall. The compliance is an important property as when a blood vessel is stretched, it stores blood volume, and then can recoil to `push` the blood flow further down the tube.

Compliance is not only regulated by passive properties; compliance can be affected by myogenic tone, as an increase in transmural pressure, such as lowering an arm below heart level, results in no change in R and a decreased C (58; 211). In opposition, decreasing transmural pressure by raising one's arm above heart level increases C without a change in R (58; 211). Only recently has it been shown that the sympathetic nervous system may affect the compliance of the blood vessel as well (33; 165; 184).

While compliance refers to the pure elastic component of the vessel wall, the vessel wall actually behaves similarly to a spring moving on a surface with friction. For perfectly elastic materials, the force developed in response to a length change is independent of the rate at which length is changed (179). However, this is not the case in the vessel wall. Vessel walls are not perfectly elastic as they do not retain their original form when a force is removed, nor do they retain the deformation, a property of plasticity (179). Therefore, the deformation will depend on both the magnitude of stress and on the rate that this stress is applied (137). Blood vessels exhibit properties appropriate to both an elastic and viscous body and therefore are termed viscoelastic (210; 211).

The viscoelastic property of the vessel wall refers to the parameter that modulates changes in compliance. Viscoelasticity is characterized by the phase difference between the applied force, or pressure, and resulting wall displacement (25). This property gives the force with which a material resists a change in its volume or diameter (37) and is partly regulated by passive elements of the vessel wall (137). This load-bearing mechanism of the vessel wall is an important parameter regulating blood flow.

When smooth muscles are rapidly stretched, they exhibit a sharp rise in tension (resistance to stretch), which then decays (stress relaxation) over time (172; 173). Siegmen (1976) noted that this resistance to stretch and resulting stress relaxation were markedly affected by the presence or absence of calcium. This calcium dependent response was not through membrane potential, alpha adrenergic receptors, or intracellular calcium, but may be due to calcium sensitivity. This myogenic response is a property of smooth muscle cells and related to the contractile elements (67). The myosin-actin crossbridge attachments may direct this mechanical response and contribute to the

resistance to stretch. When the muscle is stretched, the breaking and subsequent reformation of attachments may not generate a net force in the resting state, but may determine the magnitude of stress resistance and relaxation (173). Therefore the number of cross-bridges attached and extent of actin-myosin overlap in accordance with the sliding filament theory, may govern this response (172; 173). Recent work from our laboratory also suggests that the sympathetic nervous system may contribute to the viscoelasticity of the vessel wall (58; 116). Therefore it appears that both active and passive properties may regulate the viscoelasticity of the vessel wall.

The final parameter often studied in the context of pressure – flow relationships is the inertia of the blood and of the vessel wall (210). The mass of a fluid may be thought of as being at rest at the start of the cycle – as it is in diastole in the left ventricle (36). When the heart muscle contracts, pressure is generated, yet the fluid will resist movement because of its inertia. Fluid inertia is another form of resistance to flow, as there is a time delay to achieve the appropriate flow rate. This transient response attempts to match the flow with the pressure difference. However, due to pulsatile flow, this pressure difference is always changing and thus, the flow does not reach its appropriate rate. The higher the inertia, the longer it will take the flow to reach steady state. Inertia of blood in physiological conditions is not well studied, but is regarded as an important parameter regulating blood flow (210).

2.5 Measuring Parameters and Changes in Central and Peripheral Vascular Mechanics

Numerous models and methods have attempted to study the vasculature and the parameters regulating blood flow. However, most methods can only determine the steady state, or average of conditions. Other methods can quantify some dynamic parameters,

but are applied only within a given arterial segment. These local parameters may not be indicative of the vascular tree, central or peripheral. As previously discussed, the importance of segmenting the vascular tree is extremely valuable in detecting peripheral and central parameters as peripheral vascular mechanics dictate central recordings. Limitations occur as, historically, central vascular mechanics are studied on a global scale and local measures are limited to conduit arterial segments due to the capacity of the tools that are available. Peripheral vascular mechanics are inherently difficult to study due to the complex and small nature of these vessels. This section will summarize current methods and a novel method to study the parameters regulating blood flow in the systemic vascular, the local vessel, and vascular bed.

2.5.1 Methods for Systemic Vascular Compliance

PWV has been hailed as the best method to study vascular compliance as it is easily measured non-invasively, highly correlated to various diseases, and may serve as a marker of atherosclerosis (6; 137; 142). PWV, a measure of the speed at which the forward pressure is transmitted between two locations is used as an index of arterial stiffness (1). PWV is affected by heart rate (HR), vessel compliance, and BP (6). As HR, stiffness, and BP increase, the pulse wave travels faster due to a decreased arterial compliance. Increased PWV indicates decreased arterial compliance and systemic arterial stiffening (28).

PWV is calculated as the distance between recording sites divided by the transmission time of the arterial PP wave between the two sites (pulse wave transit time) (Figure 2.5). The pressure or flow wave, recorded simultaneously at two sites, can be recorded using pressure-sensitive transducers, applanation tonometrey, Doppler

ultrasound, and many other devices. PWV can be calculated between any two sites, such as heart to finger, heart to toe, femoral to toe, etc. The resulting PWV indicates the stiffness of the arteries between the two sites. This tool allows for us to study different areas of the systemic arterial vasculature (6).

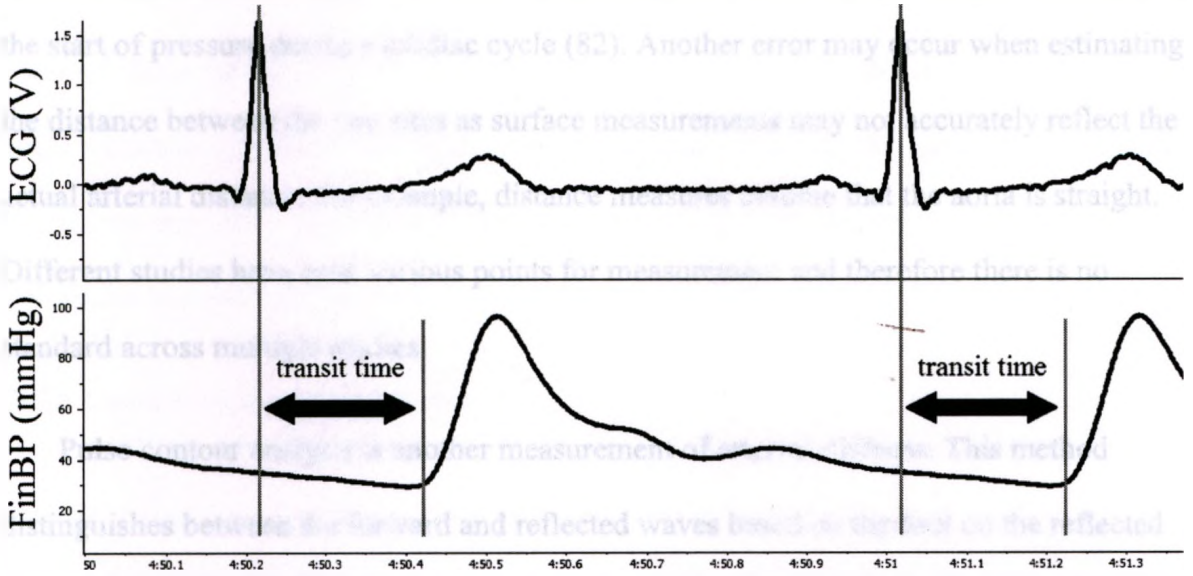


Figure 2.5 Measurement of Pulse Wave Velocity from a sample set of data. Distance is recorded as the distance between the two recording sites, the heart to finger. Pulse wave transit time is measured from the R-peak measured from the heart rate (proximal site - top panel) recorded from an electrocardiogram to the foot of blood pressure at the finger (distal site - bottom panel) recorded from the finger finometer.

Identifying the start and end of the pulse wave transit time poses some limitations to this method. Using the peak pressure may provide inaccurate timing in some conditions as the peak may be augmented by a stiffer system and therefore will exacerbate the reduced pulse wave transit time (109). The foot of the pulse wave is the most accurate location as it is unaffected by wave reflections (6; 137). Another limitation of this method is the use of the R-peak as the initiation of pressure. The true point of origin in PWV is

the initiation of pressure with ejection of blood from the heart. This does not occur directly at the R-wave but a few milliseconds after left ventricular depolarization, the so-called pre-ejection period. Heart contractility affects SV and pressure initiation, and, therefore, in conditions where heart contractility is altered, using the R-peak may over or underestimate arterial compliance (1). SV, recorded from the suprasternal notch, or cardiac velocity, both measured by Doppler ultrasound, may be a more ideal indication of the start of pressure during a cardiac cycle (82). Another error may occur when estimating the distance between the two sites as surface measurements may not accurately reflect the actual arterial distance. For example, distance measures assume that the aorta is straight. Different studies have used various points for measurement and therefore there is no standard across multiple studies.

Pulse contour analysis is another measurement of arterial stiffness. This method distinguishes between the forward and reflected waves based on the foot on the reflected wave. The time from the forward wave foot to the reflected wave foot is related to aortic PWV (109; 145) and a decrease in transit time indicates aortic stiffening. However, when conditions that alter the augmentation index such as alterations in HR, BP, heart failure, or drug therapy are not equal between groups, this tool can only be regarded as a manifestation of wave reflection and should not be used (137; 201).

Another method using an adapted Windkessel model, applies an algorithm for the diastolic portion of an arterial wave and segments central and peripheral compliance (122; 198). However, in conditions where arterial compliance is decreased and wave reflection occurs during systole rather than diastole, this analysis is inaccurate (109). Determination of the waveform is difficult because changes in the pressure pulse contour occur as the

wave propagates. Another limitation is that a pressure difference between central and peripheral arteries is not accounted for (145).

Techniques that provide information about small artery properties must be interpretted with caution. Izzo *et al.* (2001) investigated the correlation between three measures of arterial stiffness: systolic pulse contour analysis, diastolic pulse contour analysis, and cuff plethysmography (83). This study showed absent or weak correlations between these variables. In another study, although good agreement was noted between some variables (augmentation index, PWV, central and brachial PP), poor agreement was noted between other 'small artery' variables. A more accurate model is required to study peripheral vascular mechanics.

2.5.2 Local Vascular Compliance

Numerous equations exist to determine local changes in vascular compliance. Local arterial stiffness relates changes in arterial diameter to pressure changes at an arterial segment of interest (91). The changes are based on end-diastole and end-systole diameters assessed by ultrasound, whereas pressure is most accurately measured by applanation tonometry at the site of interest. Based on recommendations of an international group at the First Consensus Conference on Arterial Stiffness (145), measurements of pressure and diameter, the best indicators of local stiffness can be expressed as: Distensibility (1), Compliance (2), Peterson's Elastic Modulus (3), Strain (4), and Stress (5) (Table 2.1).

Table 2.1 Local Arterial Stiffness Equations

	Equation	Definition
1) Distensibility ($mmHg^{-1}$)	$\frac{Diameter_{SYSTOLE} - Diameter_{DIASTOLE}}{(Pressure_{SYSTOLE} - Pressure_{DIASTOLE}) * Diameter_{DIASTOLE}}$	Relative change in diameter for a given change in pressure
2) Compliance (mm/mmHg)	$\frac{Diameter_{SYSTOLE} - Diameter_{DIASTOLE}}{(Pressure_{SYSTOLE} - Pressure_{DIASTOLE})}$	Absolute change in diameter for a given change in pressure
3) Peterson's Elastic Modulus (mmHg)	$\frac{(Pressure_{SYSTOLE} - Pressure_{DIASTOLE}) * Diameter_{DIASTOLE}}{(Diameter_{SYSTOLE} - Diameter_{DIASTOLE})}$	Pressure change required for a given diameter change (Theoretical 100% stretch)
4) Strain	$\frac{Diameter_{SYSTOLE} - Diameter_{DIASTOLE}}{Diameter_{DIASTOLE}}$	Relative change in Diameter
5)Arterial Stiffness	$\frac{\ln(Pressure_{SYSTOLE} / Pressure_{DIASTOLE}) * Diameter_{DIASTOLE}}{(Diameter_{SYSTOLE} - Diameter_{DIASTOLE})}$	Ratio of logarithm (systolic/diastolic pressures) to relative change in diameter [stress to strain ratio]

However, these measurements are limited to arterial segments from which diameters can be accurately determined using ultrasonic imaging. It is essential that proper training exists for the sonographers to ensure high reliability (63; 64; 171).

Arteries are not homogenous tubes, and compliance can vary in different parts of the same vessel (164). For example, in older subjects, the decreased distensibility of the carotid artery is most pronounced in the carotid sinus (164) and therefore results in one vessel segment may not reflect the arterial system.

2.5.3 Lumped Models to Study the Entire Vascular Bed

The compliance of the small arteries is inherently difficult to study non-invasively in vivo. In the absence of adequate tools to assess the microvasculature in vivo, a model

that can be compared to measured variables is an ideal alternative (12; 210). In an attempt to segment out peripheral vascular mechanics, a lumped or Windkessel model has been used to investigate parameters regulating blood flow in the peripheral vascular bed (201). The Windkessel model is a simplification of the arterial system and describes the whole arterial system downstream of the location where measurements are obtained (201).

The first model, or the Frank two-element Windkessel model, was composed of R and C. R was calculated based on Poiseuille's law and C was determined by the elasticity of the arteries (163). Although this model did not rely solely on R and incorporated C, it was not able to predict pressure and flow during systole (201). Therefore, newer Windkessel models introduced a third component, impedance. Impedance, calculated as the wave speed times blood density divided by cross-sectional area, is another parameter regulating pulsatile flow (210). However, these models are still not adequate in describing other important components regulating blood flow (181). Other investigators have attempted to create a four-element model but the incorporation of inertance proved to be difficult and limitations existed. All of these models missed the viscoelastic property of blood flow. In 2007, Burattini and Di Salvia found a model that incorporates viscoelasticity to resolve some limitations predicted from other models (36). The incorporation of viscoelasticity allowed a new paradigm for development of a new modified Windkessel model.

2.5.4 RCKL Model

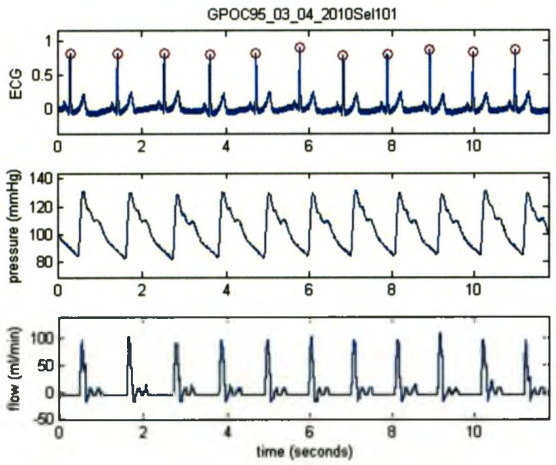
The most sophisticated, yet simplistic enough for rapid interpretation, modified Windkessel model incorporates the four elements of R, C, K, and L (211). This model provides a relation between pressure and flow and is able to predict the flow wave form

that would be produced by a given (measured) pressure wave. The calculation of a modelled waveform uses an electric circuit analogy given by total impedance (Z) of the system (Equation 1) where ω is the frequency of oscillation of the prevailing pulsatile flow and $i = \sqrt{(-1)}$ (210).

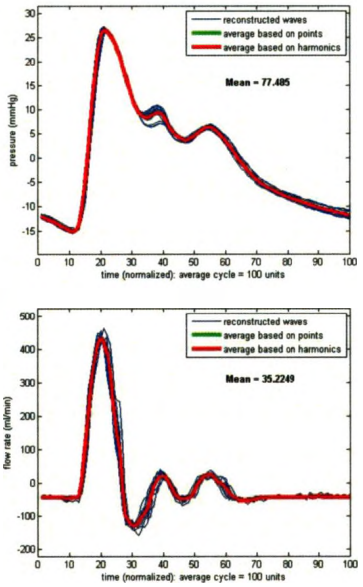
Equation 1: $$Z = \frac{R[\omega KC + i(\omega^2 LC - 1)]}{\omega C(K + R) + i(\omega^2 LC - 1)}$$

This predicted wave is then compared to the measured flow waveform to achieve congruency between the two waves. R, determined from the average pressure waveform divided by the average flow waveform, is held constant, while C, K, and L are modified until a modelled waveform is achieved that resembles the measured waveform. The most accurate model can be determined by the lowest error value that is determined by the sum of the differences squared between the two waveforms at each point along the wave (Figure 2.6). This process is automated using MATLAB programming and Fourier analysis transform techniques (34; 210; 211) (Figure 2.6).

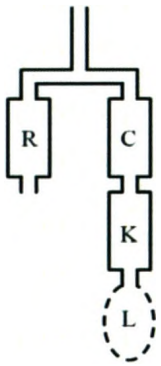
A.



B.

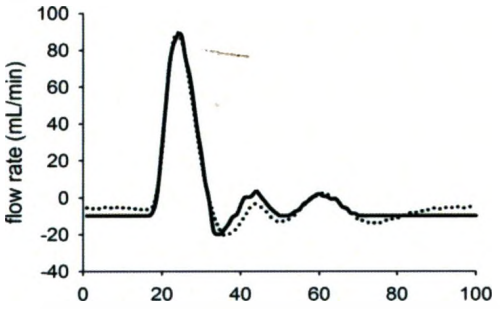


C.



$$Z = \frac{R[\omega KC + i(\omega^2 LC - 1)]}{\omega C(K + R) + i(\omega^2 LC - 1)}$$

D.



R=2.19 mmHg/mL/min
C=0.00345 mL/mmHg)
K=0.13373 mmHg/mL/min
L=7.98e-006 mmHg/mL/min^2

Figure 2.6 RCKL Model. **A.** Set of data obtained from a subject. ECG (electrocardiogram, heart rate); pressure (Finometer, brachial blood pressure); flow (Doppler ultrasound, brachial blood flow). **B.** Pressure and flow waveforms are averaged over the data set to determine Resistance. **C.** Resistance, held parallel to compliance (C), viscoelasticity (K), and inertia (L) all in series. Resistance determined from average pressure and flow waveforms is held constant while C, K, and L are modified. **D.** A final modeled (dashed) is achieved that is congruent to the measured (solid) flow waveform and values of C, K, and L are obtained (210; 211).

Examinations of the central or large arterial vascular mechanics dominate the clinical literature. This is due to the limitation of our knowledge understanding the dynamics of the peripheral vasculature. While the contributions of C have been studied locally or systemically, pulsatile vascular mechanics are also important and the relevance of such a model is valuable to detect alterations in the dynamic components of blood flow in isolated vascular beds. With this new model, we will be able to detect alterations in peripheral dynamics using the RCKL model. Attempts to investigate peripheral vascular mechanics, based on this new model, in disease states, have not been investigated.

2.6 Interim Summary

Vascular research has focused on the central, large elastic mechanics regulating BP and blood flow. This limits our understanding of vascular mechanics as many changes are expected to develop in peripheral vascular beds. This review has demonstrated that the neurogenic, myogenic, signalling molecules, and geometry of the central and peripheral blood vessels are in fact different and therefore it is essential to understand both the steady and oscillatory dynamics of the entire vascular tree. Additionally, the alterations to the arterial tree and especially to the peripheral vascular mechanics in vascular disease are not well known.

The inadequate understanding of the periphery is due to the limitations of studying these small vessels in vivo. Recently our lab has developed a model to study the peripheral vascular bed using a mathematical Windkessel model. This model incorporates the R, C, K, and L of the downstream vasculature. These parameters are regulated by both the passive and active properties of the blood vessel. The ability to detect changes in these parameters, namely R, C, K, and L may help improve detection of vascular disease.

2.7 Vascular Disease

Vascular disease, characterized by factors that alter the structure and/or function of arteries, is the leading cause of death in the general population (153). Vascular disease is a generic term and a form of cardiovascular disease that affects the blood vessels (137). Vascular disease often arises from atherosclerosis, the presence of arterial wall thickening and loss of elasticity, which characterize the presence of these vascular remodelling alterations (71).

Changes in arterial structure and anatomy affect the areas discussed in the previous sections. Various pathophysiological changes occur through the progression of vascular disease and are well documented. Although these changes in the vessel wall are well characterized during the progression of vascular disease, the alterations in the peripheral vascular bed dynamics have not been studied. The importance of investigating the properties regulating blood flow in the periphery is demonstrated by the mechanisms by which antihypertensive drugs act. These drugs decrease arterial stiffness through an effect on small arteries (194; 194) and therefore the periphery represents another important area to study.

2.7.1 Risk Factors for Vascular Disease

As one ages, manifestations of vascular disease become prominent (100; 126). However, young individuals appear to be protected from age related risks of developing vascular disease. Although the initiation of this age-associated risk is difficult to pinpoint, it appears that this risk begins in one's forties (41; 143; 144). Other risk factors negate the 'protection effect' in the young and/or accelerate the rate of age-associated risks. One of the most important advances in diagnosis has been the identification of these major risk

factors for cardiovascular diseases, which arose from large prospective cohort studies such as the Framingham Heart Study and the Seven Countries Study (94; 205).

The number one preventable risk factor for vascular disease is cigarette smoking. Other major modifiable risk factors include high BP, high cholesterol and fat levels, diabetes mellitus, and a sedentary lifestyle combined with obesity (71). Uncontrollable risk factors such as family history and gender can also affect an individual's likelihood of developing vascular disease. Men are two to three times more vulnerable than premenopausal women. However, postmenopause, women no longer exhibit advantages over men (96).

2.8 Smoking and Vascular Disease

In Canada, seventeen percent of all deaths in 2002 were attributable to smoking and twenty nine percent of all smoking-related deaths were due to heart disease and strokes (157). Beyond the already well-established strong causative relationship with cancer, smoking increases the risk for vascular disease (206). Smoking may directly or indirectly act on various risk factors contributing to the development of vascular disease and represents an interesting population for studying vascular disease.

Epidemiologic studies have identified smoking as a major cause of preventable cardiovascular mortality (188). One of the main adverse consequences of smoking is the progression of atherosclerosis and cardiovascular disease (78; 84; 164). Howard *et al.* (1998) determined that cigarette smoking was associated with a 50% increase in the progression of atherosclerosis (78). The risk of vascular disease increases with the number of cigarettes smoked (10; 51; 133; 148; 149). However, not all studies have determined this relationship and even low-tar and light cigarettes have been shown to

increase the risk of cardiovascular events (13; 21). Ultimately, cigarette smoking is associated with an elevated risk of mortality (84). By the age of 60, the risk of having severe carotid atherosclerosis for a person who had smoked 40 years, was approximately 3.5 times more compared to an individual who had never smoked (203) (Figure 2.7).

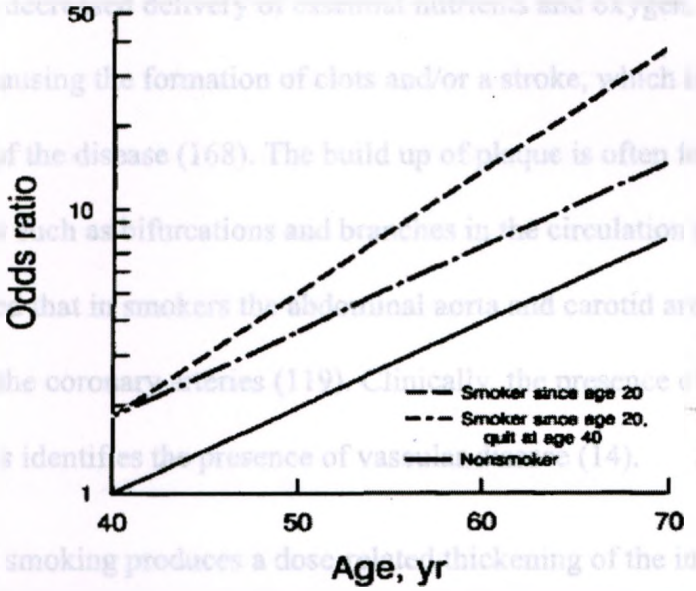


Figure 2.7 Calculated adjusted odds ratio for likelihood of having severe carotid atherosclerosis as function of age and years of cigarette smoking. Odds ratio of 1 was arbitrarily assigned to 40-year-old person who had never smoked. (203)

2.8.1 Progression of Vascular Disease

The importance of hemodynamic forces in the development of vascular disease is documented by the presence of atherosclerosis. Atherosclerosis is a progressive disease associated with the accumulation of lipid, cellular metabolites, and thickening of the intima in vessel walls (44). An IMT in the carotid artery of greater than 1mm is characterized as thickening and patients with a mean IMT of over 1.15 mm have a 94% likelihood of developing coronary artery disease (89). Intima thickening and plaque

formation is a response due to increased inflammatory markers present in the systemic vasculature (108).

Excessive intima thickening gives rise to the formation of atherosclerotic lesions which cause degeneration of the artery walls. This plaque formation results in obstructed flow and thus decreased delivery of essential nutrients and oxygen. Finally, the plaque may rupture causing the formation of clots and/or a stroke, which is unfortunately often the first sign of the disease (168). The build up of plaque is often found in areas of disturbed flow such as bifurcations and branches in the circulation (137; 179). McGill (1988) reported that in smokers the abdominal aorta and carotid arteries were more affected than the coronary arteries (119). Clinically, the presence of plaque build-up and atherosclerosis identifies the presence of vascular disease (14).

Cigarette smoking produces a dose-related thickening of the intima-media of the arterial vasculature and heavy smokers exhibit significantly increased IMT in the carotid (150) and coronary arteries (9; 10) compared to non-smokers. Cigarette smoking increases vascular inflammatory markers related to increased fat, cholesterol, and plaque build up at the arterial wall (23; 26). The increase in IMT and inflammatory markers are evident in smokers who smoke light or regular cigarettes demonstrating the negative effect of all cigarette types (13).

2.8.2 Changes in Vascular Mechanics in Smokers and Vascular Disease

The presence of atherosclerosis in the systemic vascular system is not the only documented alteration present in vascular disease. Arterial vessel walls are the primary site of disease and represent a target for demonstration of functional and structural alterations (115; 166). Arterial stiffness denotes alterations in the mechanical properties

of arteries and has become one of the most popular studied characteristics in disease states. Arterial stiffness is a broad term incorporating both the compliance and distensibility of the arterial vessels.

Arterial stiffness increases the risk and presence of vascular disease due to the stress on the vessel wall. Increased stiffness decreases the buffering capacity of the arterial tree and thus increases the afterload on the heart (109). In patients with hypertension, large artery stiffness indicates the presence, but also the severity and extent of atherosclerotic disease (28). Increased PWV is perhaps the easiest and most widely studied marker of increased systemic stiffness (74). PWV is associated with the presence and extent of atherosclerosis and vascular disease.

As discussed in Section 2.4.1, in a stiffer system, reflected waves arrive during systole rather than diastole, and BP is increased. The augmentation index, describes this augmented systolic pressure and alongside carotid IMT is associated with a high cardiovascular risk (178). PP, calculated as the difference between SBP and DBP, is often elevated in conditions of vascular disease (59; 112) affecting the compliance and distensibility of the blood vessel. Overtime, this constant baroreflex resetting and increased stress on the vessel walls leads to vascular remodelling and even stiffer vessels (93).

Acutely, smoking one cigarette decreases carotid and brachial distensibility and compliance (54; 97) and increases PWV (95; 98; 204). Chronically and acutely, smokers exhibit elevated SBP, DBP, MAP, and PP and augmentation index (54; 95; 97; 104; 107; 112; 156). Jatoi *et al.* (2007) found a direct linear relationship between smoking status

and arterial stiffness, with former smokers having intermediate values between current smokers and non smokers (87). However, other studies have not identified chronically elevated PWV, decreased compliance and distensibility, or elevated pressure in smokers compared to non-smokers (97; 112; 156; 195). The discrepancy in results may be accounted for by heterogeneous groups of subjects such as various age ranges, grouping males and females together, and/or the lack of control for confounding variables of other risk factors such as elevated BP, weight, and most importantly the location of the arteries studied. The significant variation in the designs and conditions of these studies makes direct comparisons difficult. In addition, no comparison has been made between central and peripheral vascular mechanics to our knowledge.

2.8.3 Mechanisms of Vascular Disease Progression in Smokers

Cigarette smoke expose humans to a wide range of harmful substances, several of which have the potential impact on the process of vascular disease. At least 4700 constituents of cigarette smoke have been identified, yet many more components are unidentified (65; 150). The identified components that are most often studied are: 1) carbon monoxide; 2) other vapour phase components; 3) tar; and 4) nicotine (175). For the purpose of this review, the effects of carbon monoxide, vapour phase components such as free radicals, and nicotine will be discussed.

Carbon monoxide passes easily from the lungs into the blood stream and binds to haemoglobin thereby displacing oxygen. The main mechanism by which carbon monoxide affects vascular mechanics is the resulting hypoxic environment. Hypoxic conditions have been shown to increase sympathetic nervous system activity in humans (72; 176; 180). Studies in rabbits and monkeys have reported that exposure to carbon

monoxide produced an accumulation of cholesterol in the aorta and coronary arteries which was implicated in the process of atherosclerosis (7; 8). However, acute exposure did not affect plasma lipoprotein levels, mean HR, or catecholamine production (103). Additionally, maximal aerobic capacity and exercise tolerance are reduced by carbon monoxide. Therefore, carbon monoxide may have indirect effects on the vasculature due to weight gain and decreased aerobic capacity (118).

The vapour phase of cigarette smoke contains free-radicals which increase vascular superoxide production (154). Reactive oxygen species react with nitric oxide to decrease nitric oxide and prostacyclin availability (147; 152). This decreased bioavailability of nitric oxide and prostacyclin contributes to impaired vasodilation and endothelial function (20; 99; 141). Endothelial dysfunction, an early phenomenon in vascular disease, has been observed in smokers compared to non-smokers (42; 156; 204; 213). Endothelial dysfunction, the inability of the vessel wall to dilate in response to shear stress, is a consequence of decreased synthesis or increased degradation of nitric oxide and is present in diseased states (134). Mechanisms for decreased nitric oxide production include alterations in nitric oxide synthase enzyme substrate activation, changes in the endothelial nitric oxide synthase expression, structure, and activation, and the presence of reactive oxygen intermediates that inactivate nitric oxide (136).

Flow dependent dilation, a non-invasive marker of endothelial function, is progressively decreased with increasing plaque development on the arteries (213). In smokers, over the entire range of wall thickening, arterial segments exhibited even further reductions in dilator responses compared to non-smokers (213). Evidence of endothelial dysfunction and impaired nitric oxide-mediated dilation is apparent in smokers (121;

204). However, when smokers are given nitrorusside (80), methacholine, (121) or glyceroltrinitrate (204) as vasodilators, they were able to dilate to the same extent as non-smokers. Therefore smokers exhibit a reduction in basal, but not stimulated nitric-oxide mediated vasodilation.

Nicotine is often documented as the most harmful substance of cigarette smoke and is believed to be primarily responsible for the hemodynamic effects of cigarette smoking (175). Nicotine increases Q, HR, BP and decreases endothelium derived vasodilation and nitric oxide availability (117). Nicotine and its metabolites also act as potent regulators of cell growth factors and thus contribute to the thickening of this intima caused by smooth muscle cell proliferation (40).

The response of the cardiovascular system to nicotine is mainly associated with the activation of the sympathetic nervous system (24; 198). Exposure to nicotine causes increased HR and BP via beta-2 adrenergic mechanisms and increased adrenal release of epinephrine and alpha-adrenergic mediated smooth muscle contraction. Data from Watts *et al.* (1960) indicated that nicotine is one of the most effective compounds for releasing epinephrine from the adrenal glands in dogs (198). A chronic increase in sympathetic nervous system activity resulting in vasoconstriction or decreased compliance and may cause vascular remodelling.

Vascular remodelling may cause alterations in the geometry of the vessel wall such as increases in the degradation and synthesis of collagen and the destruction and reconstruction of elastin fibres (11; 41). Dysregulation of the 'normal, healthy' contribution of elastin and collagen leads to increased arterial stiffness and modifications

of mechanical properties (207). Increased collagen and decreased elastin contribution to the vessel wall is stimulated by a number of factors. For example, increased luminal pressure stimulates collagen production and the inflammatory response degrades the extracellular matrix by creating uncoiled, less effective collagen and broken elastin molecules (177; 207). Cross-link formation is altered making the vessel wall stiffer (41). Histological examination of stiffened arteries demonstrates this disproportionate collagen and elastin content of diseased vessels (11).

In smokers, studies have found altered elastin and collagen content in compared to non-smokers (182). Although Cox *et al.* (1984) found no effect of cigarette smoking on the concentration of collagen and elastin in the carotid or femoral arteries following acute cigarette exposure in dogs, they attributed the increased stiffness to altered cross linking or type of collagen present (46). These studies focused on the central arteries only or were not able to identify the location of the remodelling. Therefore, these are limited as we have demonstrated the importance of structural changes in the periphery as well.

Cigarette smoking also increases smooth muscle cell proliferation and migration contributing to the high risk of vascular disease (40). Smooth muscle cells undergo alterations during the progression of vascular disease as the thickening of the intima has been attributed to the proliferation of these cells that migrate from the media into the intima (159; 169). The increase smooth cell proliferation and migration may lead to an altered myogenic response that is evident in vascular disease (110).

Finally, several studies have shown that smoking is more harmful in young women than men (139; 192). Pooled data from 3 population studies including 11,474 women and

13,191 men indicate that the cardiovascular risk associated with smoking was consistently higher in women than in men (192). This increased risk was independent of age, arterial BP, body mass index, and physical activity.

The underlying mechanism for the increased risk in women compared to men may involve the antiestrogenic effect of smoking. Cigarette smoke alters estradiol metabolism, reduces estrogen levels, and hastens the onset of menopause by as much as 3 years, thus increasing the duration when women are more susceptible to cardiovascular events (18; 75; 123; 197). Due to the different absolute risk of disease observed between women and men, studies grouping both sexes into one group must be interpreted cautiously.

These pathophysiological changes and alterations in vascular mechanics help identify individuals at risk or individuals with vascular disease. However, the identification of preclinical changes in the pulsatile components of the peripheral vascular mechanics may detect those at risk and identify possible progression by which the steady and pulsatile components regulating blood flow are altered.

2.9 Reversing Altered Vascular Mechanics

While the negative consequences of vascular disease have been documented and discussed, the ability of the human body to reverse these changes is not as well studied. Some risk factors, such as aging and genetics related to vascular disease, cannot be changed; however, numerous methods to decrease the risk and development of vascular disease have been identified. Lifestyle modifications consistently recommended by health care professionals (and pertinent to this study) are exercise and smoking cessation. Whether these interventions may improve the central and peripheral vascular mechanics have yet to be elucidated.

2.9.1 Exercise Training

Regular aerobic exercise has beneficial effects on the vasculature as it decreases arterial stiffness and may decelerate the age-related increase in large-artery stiffness (61). Endurance trained individuals' exhibit higher arterial compliance and decreased arterial stiffness compared to sedentary individuals (39; 185; 208). An increased maximal aerobic capacity, or VO_2max is associated with reduced arterial stiffness (193). In middle-aged sedentary men, 3 months of aerobic training increased carotid artery compliance to similar levels observed in similarly aged endurance trained men (185). Even in the smaller radial artery, higher distensibility is observed in athletes compared to sedentary subjects (62).

Although endurance training improves arterial compliance and distensibility, resistance training appears to have the opposite effect. Cortez-Cooper *et al.* (2005) studied women over an 11 week high intensity resistance training program (45). Following the training, women exhibited increased augmentation index and carotid-femoral PWV, both indicating increased arterial stiffness (45). However, in a lower, moderate intensity resistance training program, women exhibited no change in arterial stiffness following 12 weeks of training (208).

Exercise training is associated with an improved cardiac performance and may reduce sympathetic activity (125; 132; 160). Increased pulsatile flow and stretch associated with improved endothelial signalling and nitric oxide stimulation is also evident following aerobic training (5; 29; 66). However, the timing, ability to reverse already damaged arteries, and the effects on central versus peripheral vascular mechanics are not well known.

2.9.2 Smoking Cessation

Smoking cessation at any age results in beneficial effects and decreases overall mortality and morbidity rates from cancer and vascular disease (90). The speed and magnitude of risk reduction following smoking cessation is variable, with studies reporting 3 to 20 years required for significant risk reductions (31). The overall and cardiovascular mortality among former smokers approaches the level of that of a never smoker after 10-14 years of abstinence (90). Studies have reported that former smokers of 1 to 10 years exhibit levels of arterial stiffness levels, measured by PWV and augmentation index, that fall between current smokers and non-smokers (87). After 10 years, there was no difference in arterial stiffness between former smokers and non-smokers (87). In as early as one year, smoking cessation results in decreased presence of vascular inflammatory markers (73).

While large-artery stiffness improves following smoking cessation, the development and progression of aortic atherosclerosis may persist (47). Although the relative risks for some cardiovascular risk factors are improved, there still appears to be an increased risk in former smokers compared to non smokers for atherosclerosis and vascular disease despite the number of years one is smoke free (Figure 2.7) (203; 206).

While exercise and smoking cessation improve morbidity and mortality and display beneficial results on the systemic vasculature, the short-term effects are not known. These studies have also focused on large-artery, or central, vascular mechanics and the effect of lifestyle modifications on central versus peripheral vascular mechanics have not been studied. If early changes occur first in the periphery with smoking, their vascular segments may show rapid recovery with smoking cessation.

2.10 Purpose

Based on the above background, it bears repeating that the purpose of this study was to investigate central and peripheral arterial vascular mechanics in young smokers and non-smokers. The second purpose was to determine whether these altered vascular mechanics can be improved following a 14 week intervention program of aerobic exercise and smoking cessation program.

2.11 Hypothesis

It was hypothesized that vascular mechanics will be affected detrimentally in the peripheral vascular bed of young smokers in the absence of central changes. This hypothesis predicts that, compared to non-smoking control subjects, peripheral vascular mechanics will be altered in young smokers. Despite these changes in the periphery, it is hypothesized that central vascular measurements will not be different between non-smokers and smokers. The second hypothesis was that these altered peripheral vascular mechanics can be restored in young smokers following the 14 week combined intervention program.

CHAPTER 3 : TOOLS AND TECHNIQUES

To study central and peripheral vascular mechanics, the accurate collection of arterial pressure and velocity waveforms are required. As outlined below, a combination of finger based arterial pressure measures, ultrasound imaging, and Doppler ultrasound devices were used to study vascular mechanical properties in this study.

3.1 Brachial Artery Pressure

The Finometer™ was used to collect pressure waveforms in the brachial artery and Doppler ultrasound was used to attain arterial blood flow velocity waveforms and diameter measures. The Finometer™ system obtains continuous BP waveforms from a finger cuff (Finometer; Finapres Medical Systems Amsterdam, The Netherlands). A transfer function is used to calculate the continuous BP in the brachial artery (32; 68). Previously, we demonstrated the accuracy of this calculation using hand-held applanation tonometry (211). The main components of the finger cuff are an inflatable air bladder and a plethysmograph consisting of an infra-red light source and a light detector. The air bladder is connected to the front-end unit via an air hose and both components of the infrared plethysmograph via a cuff cable (68).

Arterial pressure in the finger is measured using the volume-clamp method where the diameter of the finger arteries under the cuff is kept constant (clamped), known as the set-point. Changes in finger artery diameter are detected by means of an infrared photo-plethysmography built into the finger cuff. If during systole an increase is detected in finger diameter, the finger cuff pressure is immediately increased to prevent a diameter change (30). This finger cuff pressure is, therefore, equal to arterial pressure. From this

finger cuff pressure, an arterial brachial waveform can be calculated during each cardiac cycle.

A brachial BP waveform is reconstructed from finger pressure in three steps. First, the finger BP waveform is filtered to a brachial waveform. The systolic and diastolic levels on the filtered waveform are averaged over a 30 second period just before the upper-arm cuff is inflated, and these averaged values are used in the correction formulas. Second, the hydrostatic height correction is applied, and the shift in the entire waveform and its levels is computed. As the current study was performed in the supine position, the finger from which BP was measured was held at heart level. Third, the return-to-flow (RTF) calibration formula is applied that uses the RTF systolic pressure just measured. The RTF system was developed to provide for an individual patient calibration (32). A subject's individual RTF calibration shift is thus computed. After one successful supine RTF calibration, all pressure differences meet the requirements for proper data collection (69). The difference between the RTF calibration shift and the level correction shift at calibration time is added as a fixed and permanent shift on top of the beat-to-beat adjusted level correction and remains in place until a new RTF calibration is performed. The resulting waveform and levels are labelled RTF intrabrachial artery pressure. (68; 69).

The Finometer device also calculates a cardiac SV and cardiac output measurement, based on the arterial pressure waveform. This process, accomplished by the "modelflow" method (200), as implemented in the Finometer, computes an aortic flow waveform from either finger or intra-arterial pressure by stimulating a non-linear three-element model of the aortic input impedance. Integrating the computed aortic flow waveform per beat provides left ventricular SV and consequently Q by multiplying SV by the instantaneous

HR. The relation of cross-sectional area to arterial pressure is described by an arctangent equation with age and sex dependent parameters based on data derived from human aortas and aortic impedance (101). Accordingly, the model makes use of the individual age, sex, height, and weight as input for the aortic area-pressure relationship to be simulated. The result is continuous monitoring of Q.

The accuracy of the Finometer findings are reported in numerous articles comparing the Finometer results with intra-arterial findings (69; 81; 174).These studies have identified that the Finometer is accurate and within the Association for the Advancement of Medical Instrumentation requirements (69).The Modelflow Q calculation has been compared to thermodilution Q in patients undergoing coronary artery bypass surgery and validated for use (86). However, some limitations have been identified as the Finometer system in healthy young subjects may overestimate the brachial SBP as a consequence of PP amplification (81). However, reconstruction of intrabrachial artery pressure from finger artery pressure with waveform filtering and level correction reduces the pressure differences substantially (69). In addition, a sphygmomanometer or manual BP calibration can be used on the other arm to calibrate accurate baseline readings. Nonetheless, the Finometer device is particularly adept at tracking beat-by-beat changes in BP.

3.2 Ultrasound

Ultrasound is a tool that uses high-frequency sound waves to study the human body. Ultrasonic imaging provides an excellent modality by which the control of blood flow can be studied. The ability of ultrasound to detect continuous blood flow velocity (or blood

flow when vessel diameters are incorporated) waveforms makes this tool ideal for studying pressure-flow relationships in vascular beds.

Sound can be propagated through almost any medium. Sound has a frequency (number of oscillations per second), a speed (different speeds depending on the medium), wavelength (distance between peaks), power/intensity (rate at which energy is transported by the wave through the medium), amplitude (difference between the positive and negative peaks), displacement, and directional [(classified as longitudinal (alternating areas of compression and rarefaction)] or transverse (moving perpendicular to the sound) features (124). The term 'ultrasound' refers to any sound whose frequency is above the audible range of the human ear (137). The frequencies used in diagnostic applications range from 1.5 to 15MHz (4). At these high frequencies, the wavelength of the ultrasound (which determines the spatial resolution which can be achieved) is small. Since wavelength is inversely proportional to frequency, the best resolution is achieved at the highest frequency. However, the attenuation of the ultrasound as it passes through tissue increases exponentially with frequency and the best penetration is therefore achieved at the lowest frequency. The net result of this conflict is that, for a given application, the highest frequency compatible with the required depth of penetration is used. Thus 10MHz ultrasound can be used with very superficial vessels, such as the carotid and brachial arteries, whereas lower frequencies must be used for deep-lying vessels (4; 63; 124).

3.2.1 Ultrasonic Imaging

Ultrasound is transmitted as 'pulse-echos' that are similar to SONAR (SOund NAvigation and Ranging) and RADAR. In these techniques, pulses are transmitted along a narrow beam which is swept through an area of interest. Transmitted energy is reflected

and collected back to the point of origin. As sound travels at a specific velocity through the medium of interest (e.g., biological tissues = 1570 cm/sec) the arrival of any echo can be related directly to the depth of the scatterer producing that echo. In ultrasonic imaging, the image is built up by displaying each echo as a dot on the display screen. This intensity of the dot is determined by the strength (amplitude) of the echo, while its location is dictated by the position of the ultrasound beam and the time of arrival of the echo. By scanning the beam, a two-dimensional 'B-mode' image is built up on the display. This is the basis of both ultrasonic imaging and Doppler ultrasound. The brief pulses are produced by the piezo (pressure) electric effect. In ultrasound imaging, transducers use the same crystal to generate and receive the signal. Similarly, pulsed Doppler ultrasound uses one crystal to transmit and receive the signal. Otherwise, continuous wave Doppler ultrasound uses one crystal to continuously emit and another to receive continuously the reflected signal (124).

The piezoelectric crystal converts one form of energy into another, and in the case of ultrasound it converts electrical energy to mechanical vibration. The piezoelectric materials in the transducer will vibrate mechanically when a voltage is applied across them. The frequency of the voltage applied will affect the vibration frequency of the material. Many transducers are designed to function over a wide range of frequencies (124). Typically, the frequencies of 2.8 MHz to 15 MHz are used for cardiovascular imaging and 4-5 MHz for blood flow velocity detection. At these frequencies, ultrasound travels very poorly through air, and the transducers must be coupled to the patient through liquid, normally a gel (63). The ultrasound transmitted travels along a beam, which is determined by the frequency, size, and shape of the transducer. As the transmitted

ultrasound travels through the body, it is attenuated and scattered. The form taken by the scattering depends on the nature of the 'obstacle.' Small objects, such as red blood cells, scatter the ultrasound weakly in all directions. However, large interfaces such as the vessel wall reflect the ultrasound in a single direction similar to a mirror where the angle of reflection is equal to the angle of incidence. Therefore the vessel wall is imaged well when the ultrasound strikes it at 90 degrees as the energy returns to the receiving transducer where it is detected and converted to an electrical signal. When an ultrasound pulse returns to the transducer, it will cause the transducer to vibrate, and this will generate a voltage across the piezoelectric element. The amplitude of the returning pulse will depend on the proportion of the ultrasound reflected and the amount by which the signal has been attenuated along its path. The arrival of any echo can therefore be related directly to the depth of the scatter producing that echo (189). This is the basis of both ultrasonic imaging and Doppler ultrasound.

3.2.2 Doppler Ultrasound

A piezoelectric crystal, when energized with an appropriate electronic apparatus, transmits sound at a frequency of several million cycles per second downstream along the flowing blood. A portion of the sound is reflected by the flowing red blood cells, so that the reflected sound waves travel backward from the blood toward the crystal. However, in arterial segments, these reflected waves have a lower frequency than the transmitted wave because the red cells are moving away from the transmitter crystal. The difference in frequency between the transmitted and reflected waves is called the Doppler Effect. This is the same effect that one experiences when a train approaches and passes by while blowing its whistle. Once the whistle has passed by the person, the pitch of the sound from the whistle suddenly becomes much lower than when the train is approaching. The

transmitted wave is intermittently cut off, and the reflected wave is received back onto the crystal, and then amplified by the electronic apparatus. Another portion of the apparatus determines the frequency difference between the transmitted wave and the reflected wave, this also determining the velocity of blood flow (70).

The Doppler Effect occurs whenever there is relative movement between a sound source and a sound receiver. The observed frequency at the receiver is altered in proportion of the relative velocity between the course and receiver. The observed frequency is increased if the two structures are moving towards each other and decreased if they are receding. When ultrasound is reflected from a moving target, the frequency change can be used to detect that movement, known as the Doppler shift frequency (Equation 2).

Equation 2: $f_D=2f_o(v\cos\theta)/c$;

where f_o is the ultrasound frequency, v is the speed of movement of the scatterer, θ is the angle between the direction of movement of the scatterer and the ultrasound beam, and c is the speed of sound propagation in the scattering medium (1570m.s for blood) (137).

When ultrasound with a known frequency (f_o) is scattered by an object moving towards the transducer, the received echo will have a frequency which is slightly higher than f_o. Conversely, an object moving away from the transducer will cause a shift to a lower frequency. The amount of this Doppler shift is determined by the original frequency transmitted, and by the speed and direction of movement of the scattered relative to the ultrasound beam. As is virtually always the case, the Doppler shift falls within the audible range of the human ear. Therefore with sufficient experience, it is

possible to recognize the changes of sound associated with poor quality, and even stenosis or turbulence (4).

To detect blood vessels, it is seldom possible to direct the beam along the long axis of the vessel and there is usually an angle between the direction of the beam and the direction of blood movement. In Doppler ultrasound, the Doppler shift actually occurs twice, first when the sound strikes the target and second when it is reflected. Referring to the equation, it is evident that the angle of insonation strongly affects the Doppler shift. When the ultrasound beam is at right angles to the vessel (cos 90 degrees = 0), the Doppler shift frequency is zero and no signal will be obtained. At angles approaching 90 degrees, the ability to discriminate between forward and reversed flow is impaired. Typically at such angles, both positive and negative Doppler shifts are detected, appearing equally above and below the zero-flow line. Angles exceeding 60 degrees are unsuitable for quantification of flow, since the rate of change of the cosine function increases rapidly toward 90 degrees so that small errors in angle measurement cause large errors in angle-corrected velocity computations. In contrast, when the transducer is aligned with a vessel (cos 0 degrees =1), the maximum Doppler shift will be obtained and angle uncertainty has its least effect. However, there are technical difficulties in obtaining signals at such low angles because of total reflection of sound waves at vessel walls. For these reasons, angles between 30 and 60 degrees are usually employed (187).

3.2.3 Validity

Ultrasonic imaging provides an excellent modality by which to study the control of blood flow. It is a non-invasive tool that can obtain beat-to-beat vessel diameters and as well uses Doppler ultrasound to obtain blood flow velocity.

In support of other studies demonstrating the validity of ultrasound to study blood flow (63; 130; 187), Shoemaker and colleagues (1996) demonstrated that Doppler ultrasound measures of arterial diameter and blood velocity were reproducible across different test days during both rest and exercise conditions. Therefore ultrasound can be used as a reliable, non-invasive means of measuring blood flow in repeated-measures designs (171).

CHAPTER 4 : METHODS

All experimental procedures were approved by the Health Sciences Research Ethics Board at The University of Western Ontario Ethics Committee for Research on Human Subjects (Getting Physical on Cigarettes, Review #16306 and Normative Vascular Indices Across the Lifespan, Review #16921 Appendix 1). Participants provided informed written consent for this study.

4.1 Subjects

Seventy-eight women (age range was 18 to 40 years, Table 5.1, Section 5.1) volunteered to participate. Fifty- three women participated in the Getting Physical on Cigarettes Program. These 53 women were smokers, defined as individuals who smoked more than 10 cigarettes per day for the past 2 years. In addition, twenty-five non-smokers, defined as women who had never smoked, served as the control group. Participants were requested to abstain from eating, exercise, alcohol, coffee, tea, caffeinated soft drinks and chocolate 3 hours before testing. Based on responses to health questionnaires, no participant was being treated for cardiovascular or neurological disease, allergies, and none consumed medications contraindicative to participation. There was no standard timing of measurements relative to the use of oral contraceptives or menstrual cycle.

4.2 Recruitment

Recruitment of participants was accomplished through numerous outlets: Advertisements were placed in local (London, Ontario) newspapers, emails were sent to The University of Western Ontario community, and posters were placed on campus. Interested participants responded to the recruitment if interested in participating. Initial screening took place to determine eligibility.

4.3 Protocol Design

The first part of this study was designed to investigate the central and peripheral vascular effects of smoking compared to non-smoking control women. The second part of this study was designed to investigate the impact of smoking cessation and aerobic exercise on central and peripheral vascular mechanics. All participants underwent the same protocol to assess these parameters. Measurements of vascular health were performed in the Laboratory for Brain and Health (Health Science Building Room 402) and the Neurovascular Research Lab (Thames Hall Room 3110) as both locations had the same equipment. Participants in the smoking groups were tested twice, once at baseline and again after the fourteen week intervention described below. The control group was tested only once to provide reference data. All tests were performed in the exact same manner, setting, and design.

After arriving, participants were acquainted with the lab set-up and protocol. HR was determined from a standard three-lead electrocardiogram (ECG). Respiration was monitored by a respiratory belt. The pressure waveform at the foot was measured using a pulse oximeter placed around the hallux (large toe). Arterial BP was monitored continuously from the middle finger of the right hand by photoplethysmographic methods from which pulsatile brachial pressure was determined (Finometer; Finapres Medical Systems Amsterdam, The Netherlands). The accuracy of the calculated shape of the calculated brachial BP waveform has been previously confirmed in our laboratory (211). Automated measures were adjusted to the average of three sphymogameter BP measures (BPTru). A second cuff was placed on the brachial artery on the right arm. After a two minute recording period, a RTF calibration was performed to individually calibrate the upper-arm pressure with the finger pressure. Following calibration and BP measurements,

each participant rested quietly for five minutes to collect continuous ECG, finger and brachial BP, Q, and SV onto Lab Chart (v. 7.0 ADInstruments; Colorado Springs).

After the resting period, ultrasound and Doppler imaging was performed on the carotid and brachial arteries (10 MHz transducer, GE Vivid 7 and 4MHz Doppler Probe, Multigon Inc.). The carotid artery was imaged approximately 2 cm proximal to the carotid bifurcation. The brachial artery was imaged approximately 4 to 6 cm proximal to the medial epicondyle. A 2D longitudinal B-mode image of each artery was obtained which clearly showed the anterior and posterior walls. Images were stored (Echo PAC™ Dimension system) for offline analysis. Blood flow velocity was obtained using Doppler ultrasound (4 MHz, GE Vivid 7). For each location, the spectral mean velocity was collected and recorded for at least one minute.

Analog signals for hemodynamic and ultrasound variables were sampled at 1000Hz and collected with an online data acquisition and analysis package (Powerlab; ADInstruments).

4.4 Lifestyle Intervention – Aerobic Exercise and Smoking Cessation

Eligible women who smoked were asked to take part in a supervised exercise program three times per week for a total of fourteen weeks. Each exercise session consisted of a cardiovascular work-out on treadmills, stationary bikes, rowers, and/or a stepper, for 45 minutes. Participants wore a HR monitor (Polar Electro, USA) and were asked to exercise at an exercise intensity that was based on a predetermined stress test to volitional fatigue. The participants' workload progressively increased until they were consistently exercising at a training intensity of 70-75% of their estimated HR max. The exercise intensity is classed as vigorous and this type of exercise delivery has been used in previous research

and was related to successful short-term (151) and long-term smoking abstinence (113). Any missed sessions were made up at another time.

After four weeks of the exercise program, subjects were asked to quit smoking completely. To assist participants, nicotine replacement therapy (NICODERM ®) was given to each participant. These daily patches were placed on the skin. Doses depended on each individual's level of smoking, but followed the recommended guidelines based on the Nicoderm®, 3 step, 10 week program: 1) A 21mg patch each day for 6 weeks; 2) A 14mg patch each day for 2 weeks; 3) A 7mg patch each day for 2 weeks, for a total of 10 weeks. Smoking cessation was confirmed by weekly expired carbon monoxide tests which occurred at random once per week for the 10 weeks.

Upon completion of the fourteen week intervention (including the ten weeks of smoking cessation and nicotine replacement therapy described above), participants returned to the lab for follow-up testing.

4.5 Data analysis

HR, SBP, DBP, Q, SV, and MAP were averaged over the five minute resting period at the start of each protocol. Pressure waveforms, Q, and SV were calculated from the modelflow method on a beat-by-beat basis from the BP waveform (200). TPR was calculated from the MAP divided by Q.

4.5.1 Vascular Mechanics

4.5.1.1 Pulse Wave Velocity

Pulse wave transit time was measured as the time from the upstroke of velocity from the carotid velocity measured from Doppler ultrasound to the diastolic foot of pressure measured at the finger (Finometer) or the foot of pressure measured at the toe (pulse

oximeter). PWV was measured using the distance from the heart to finger or toe, divided by the pulse wave transit time to the finger or toe.

4.5.1.2 Local Conduit Vessels

Arterial diameters were made from 2D B-mode images taken in the long-axis plane. Diameter measurements were made at end-systole and end-diastole. Three measurements were taken: 1) From outer edge of the anterior wall to inside the posterior wall (including the intima-media); 2) From the inner edge of the anterior wall to inside the posterior wall (including the intima-media); 3) From the outside of the intima-media of the anterior wall to inside the posterior wall (including the intima-media) (Figure 4.1). This was repeated three times across (right, middle and left side) the entire imaged vessel. The three values were then averaged to determine: Wall thickness = (2-1); Intima-Media Thickness = (3-2); and Diameter = (2). The equations for Distensibility, Compliance, Peterson's Elastic Modulus, Strain, and Arterial Stiffness are given in Section 2.5.1.1 Table 2.1.

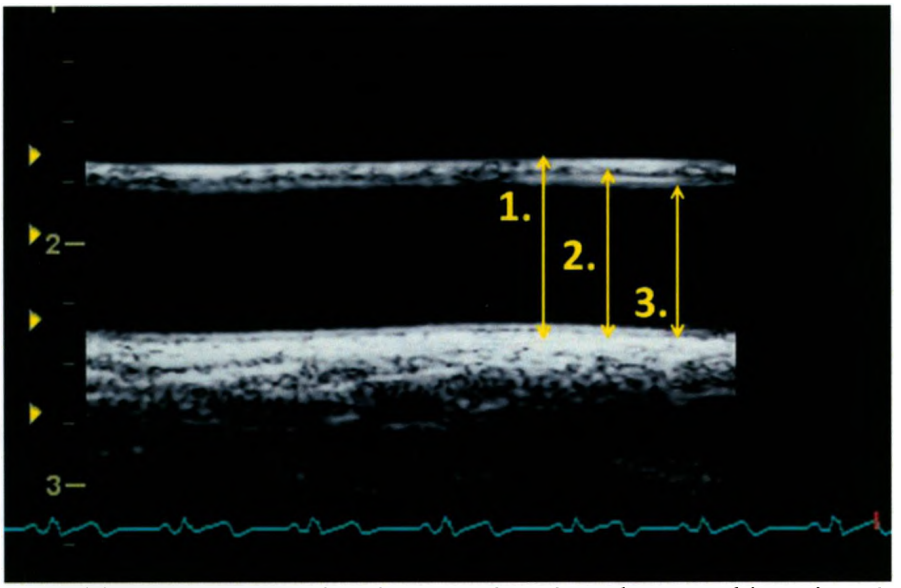


Figure 4.1 Carotid Artery Image taken in B-mode using ultrasound imaging. 1. From outer edge of anterior wall to inside the posterior wall (including the intima-media); 2. From the inner edge of the anterior wall to inside the posterior wall (including the intima-media); 3. From the outside of the intima-media of the anterior wall to inside the posterior wall (including the intima-media).

4.5.1.3 Peripheral Forearm Vascular Mechanics

Blood flow was calculated as the product of diastolic artery cross-sectional area and the instantaneously recorded blood velocity. Cross-sectional area was calculated using the end-diastole diameter obtained from the average of three measurements of vessel diameter from the ultrasound 2D B-mode image.

Peripheral R, C, K, and L values were determined using the lumped Windkessel model (and discussed in Section 2.5.3) (211). The value of vascular R was determined from simultaneous measurements of mean pressure (Finometer) and mean flow (Doppler Ultrasound) at the point of entry to the forearm vascular bed. In the same way, the values of C, K, and L in a vascular bed can be determined from simultaneous measurements of the oscillatory components of pressure and flow at the point of entry. The calculations of C, K, and L are based on a modified Windkessel model, where C, K, and L, arearranged in series and are in parallel to R. After determining R, this value is entered into the

impedance formula (Section 2.5.3 Equation 1), whereby C, K, and L are estimated until a calculated waveform is congruent to the measured waveform.

Ten congruent, similar waveforms of ECG, brachial pressure, and brachial flow were obtained from each subject. These data were saved in MATLAB format to be entered into the RCKL program. Due to the unknown shift during calculation of brachial pressure from the finger, several datasets are saved with different shifts. The correct shift was determined by the estimated waveform that gave the lowest error, calculated as the square of the difference between the estimated and measured waveforms at each point along the curve. The values of C, K, and L that give the lowest error, were deemed the 'best' fit. It is important to note that an exact fit cannot be determined as the model is a simplified representation of the vascular system.

4.5.2 Arm Volume

Arm volume was measured to address the potential impact of arm size on the measured variables. This value was calculated from measurements of arm length (wrist to elbow) and arm circumference taken at the wrist and elbow, assuming a conical shape to the arm, and using the equation:

$$[\text{Length} * ((\text{wrist}^2 + (\text{wrist}*\text{arm}) + (\text{arm}^2))]/(12/3.14)$$

4.6 Statistical Analysis

To address the effect of smoking compared to non-smoking control subjects, data were analyzed using a Mann-Whitney non parametric test (SPSS Statistics 18). The effect of the intervention program was assessed using a paired t-test (SPSS Statistics 18). Post-hoc Mann-Whitney analysis was performed to compare post-intervention subjects to non-

smokers (control). To address interactions of age and menstrual cycle, post-hoc ANCOVA analysis was performed. Shapiro-Wilk tests were performed to determine if the data were normally distributed (SPSS Statistics 18).With all analyses, the probability level was $p<0.05$. Data are expressed as means ± standard deviation.

CHAPTER 5 : RESULTS

BASELINE RESULTS – NON-SMOKERS (Controls) and SMOKERS

5.1 Systemic Haemodynamics

Although within a normal, heathy range, SBP, DBP, and MAP were higher in the smokers compared to non-smokers ($p<0.05$) (Table 5.1).

Table 5.1 Subject Characteristics

	Non-Smokers	Smokers
N	25	53
Age (years)	25.8 ± 5.3	27.7 ± 5.6
Height (cm)	167.4 ± 6.4	164.4 ± 6.7
Weight (kg)	63.2 ± 12.0	70.2 ± 15.8
SBP (mmHg)	108 ± 7	117 ± 9*
DBP (mmHg)	63 ± 6	67 ± 8*
PP (mmHg)	45 ± 7	49 ± 9
MAP (mmHg)	78 ± 6	84 ± 7*
HR (bpm)	69 ± 12	75 ± 10
Q (L/min)	5.3 ± 1.4	5.9 ± 1.4
TPR (mmHg/L/min)	17 ± 5	15 ± 4

Values are mean ± standard deviation (SD). SBP, systolic blood pressure; DSP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; Q, cardiac output; TPR, total peripheral resistance.
* Indicates $p<0.05$ for Smokers vs. Non-smokers.

5.2 Central Vascular Mechanics

5.2.1 Pulse Wave Velocity

PWV to the finger was not different between smokers (1363.02 ± 204.13 cm/s) and non-smokers (1428.82 ± 198.75 cm/s) ($p=0.227$). PWV to the toe was not different between smokers (950.91 ± 170.90 cm/s) and non-smokers (956.36 ± 143.69 cm/s)($p=0.829$).

5.2.2 Carotid Artery Characteristics

Carotid wall thickness was not different between smokers (0.60 ± 0.12 mm) and non-smokers (0.65 ± 0.14 mm) (p=0.122). Carotid IMT was not different between smokers (0.42 ± 0.09 mm) and non-smokers (0.41 ± 0.08 mm) (p=0.352).

5.2.3 Local Carotid Mechanics

Local carotid measures of arterial stiffness and vascular characteristics are presented in Table 5.2. No significant differences occurred between groups.

Table 5.2 Local Carotid Mechanics

	Strain	Arterial Stiffness	Peterson's Elastic Modulus (mmHg)	Distensibility ($mmHg^{-1}$)	Compliance (mm/mmHg)
Non-Smokers	0.09 ± 0.02	6.56 ± 2.10	548.64 ± 180.14	$1.97 \times 10^{-3} \pm 0.55 \times 10^{-3}$	$1.23 \times 10^{-2} \pm 0.36 \times 10^{-2}$
Smokers	0.10 ± 0.03	6.41 ± 2.61	568.31 ± 209.54	$1.99 \times 10^{-3} \pm 0.74 \times 10^{-3}$	$1.21 \times 10^{-2} \pm 0.43 \times 10^{-2}$

Values are mean ± standard deviation (SD).

5.3 Peripheral Vascular Mechanics

5.3.1 Brachial Artery Characteristics

Brachial wall thickness was not different between smokers (0.36 ± 0.07 mm) and non-smokers (0.38 ± 0.16 mm) (p=0.195).

5.3.2 Local Brachial Mechanics

Local brachial measures of arterial stiffness and vascular characteristics are presented in Table 5.3. Compared to non-smokers, Arterial stiffness and Peterson's Elastic Modulus were higher in smokers ($p<0.05$), whereas Distensibility and Compliance were lower in smokers ($p<0.05$). Compliance normalized to arm volume was also lower in smokers compared to non-smokers ($p<0.05$).

Table 5.3 Local Brachial Characteristics

	Strain	Arterial Stiffness	Peterson's Elastic Modulus (mmHg)	Distensibility ($mmHg^{-1}$)	Compliance (mm/mmHg)	Compliance normalized to Arm Volume ($mmHg^{-1}/cm^3$)
Non-Smokers	0.04 ± 0.02	17.54 ± 10.29	1451.45 ± 798.08	$8.86 \times 10^{-4} \pm 4.06 \times 10^{-4}$	$2.85 \times 10^{-3} \pm 1.34 \times 10^{-3}$	$3.92 \times 10^{-7} \pm 2.02 \times 10^{-7}$
Smokers	0.03 ± 0.02	24.12 ± 15.80 *	2158.98 ± 1471.93 *	$6.41 \times 10^{-4} \pm 3.59 \times 10^{-4}$ *	$2.18 \times 10^{-3} \pm 1.27 \times 10^{-3}$ *	$2.84 \times 10^{-7} \pm 1.58 \times 10^{-7}$*

Values are mean ± standard deviation (SD).
*Significant difference, $p<0.05$ for Smokers vs. Non-smokers

5.3.3 Peripheral Forearm vascular properties

Brachial MAP was increased in smokers (91 ± 11 mmHg) compared to non-smokers (77 ± 8 mmHg) ($p<0.001$). Brachial blood flow was similar in smokers (20 ± 11 mL/min) and non-smokers (24 ± 10 mL/min) ($p=0.122$). Forearm vascular R was increased in smokers (5.73 ± 3.12 mmHg/mL/min) compared to non-smokers (3.99 ± 2.39 mmHg/mL/min) ($p<0.01$) (Figure 5.1). Compared to non-smokers ($7.27 \times 10^{-3} \pm 3.19 \times 10^{-3}$ mL/min), forearm vascular C was decreased in smokers ($4.11 \times 10^{-3} \pm 1.84 \times 10^{-3}$ mL/min) ($p<0.001$). After normalizing forearm vascular C to arm volume, C was still lower in smokers ($5.17 \times 10^{-6} \pm 2.54 \times 10^{-6}$ mL/min/cm^3) compared to non-smokers ($9.87 \times 10^{-6} \pm 4.22 \times 10^{-6}$ mL/min/cm^3) ($p<0.001$). Forearm vascular K was increased in smokers (0.20 ± 0.09 mmHg/mL/min) compared to non-smokers (0.11 ± 0.05 mmHg/mL/min) ($p<0.001$). Forearm vascular L was increased in smokers ($3.34 \times 10^{-5} \pm 4.21 \times 10^{-5}$ mmHg/mL/min^2) compared to non-smokers ($1.39 \times 10^{-5} \pm 1.71 \times 10^{-5}$ mmHg/mL/min^2) ($p<0.05$) (Figure 5.2).

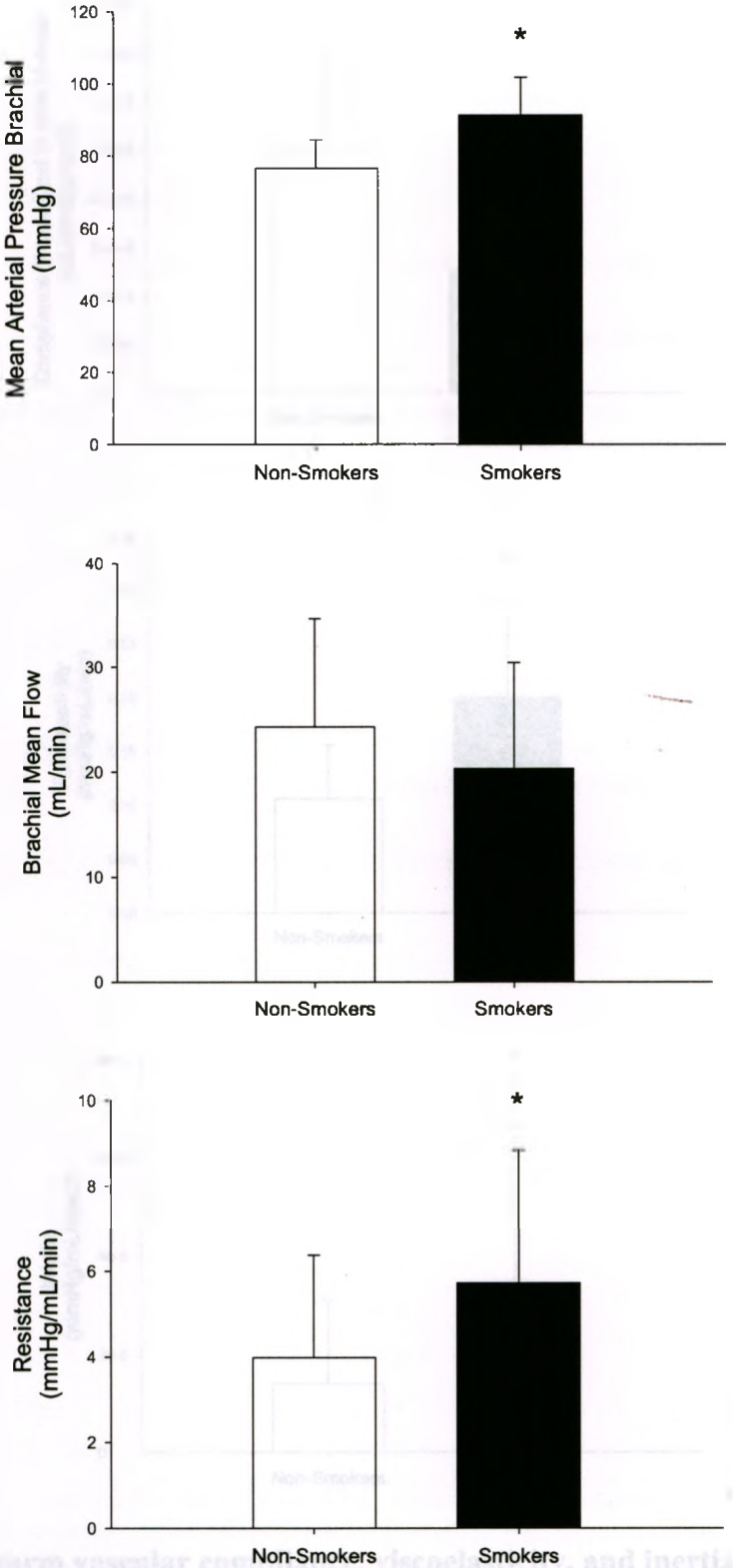


Figure 5.1 - Forearm vascular mean arterial pressure, flow, and resistance in non-smokers (white) and smokers (black).*Significant difference between groups $p<0.01$.

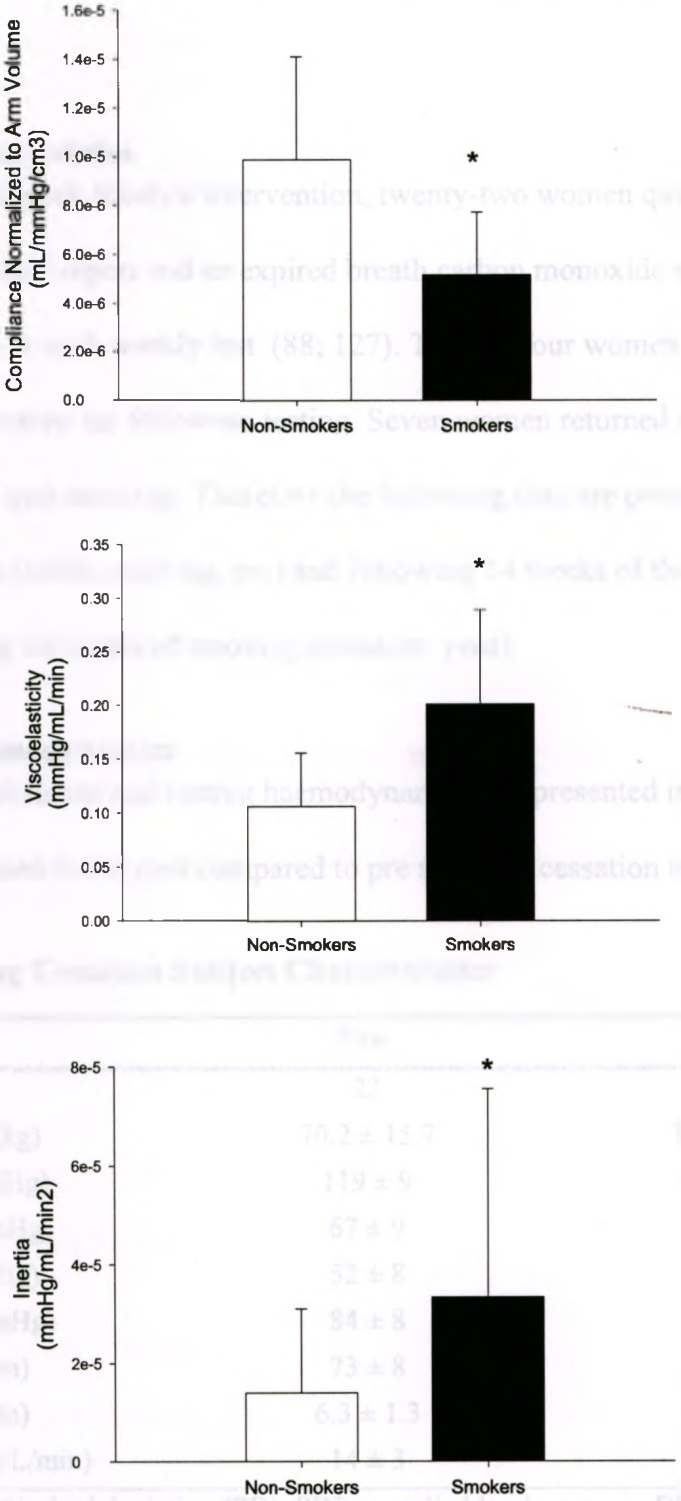


Figure 5.2 Forearm vascular compliance, viscoelasticity, and inertia in non-smokers (white) and smokers (black).*Significant difference between groups $p<0.05$.

LIFESTYLE INTERVENTION RESULTS – SMOKERS (Pre) and QUITTERS (Post)

5.4 Subject Characteristics

Following a 14 week lifestyle intervention, twenty-two women quit smoking which was confirmed by self-report and an expired breath carbon monoxide reading of less than 6 parts per million at each weekly test (88; 127). Twenty-four women were lost in the study and did not return for follow-up testing. Seven women returned for follow-up testing but did not quit smoking. Therefore the following data are presented for these 22 women at baseline (while smoking, pre) and following 14 weeks of the intervention program (including 10 weeks of smoking cessation, post).

5.5 Systemic Haemodynamics

Subject characteristics and resting haemodynamics are presented in Table 5.4. SBP and HR were reduced in the post compared to pre smoking cessation tests ($p<0.05$).

Table 5.4 Smoking Cessation Subject Characteristics

	Pre	Post
N	22	22
Weight (kg)	70.2 ± 15.7	75.6 ±14.6*
SBP (mmHg)	119 ± 9	113 ± 9*
DBP (mmHg)	67 ± 9	64 ± 8
PP (mmHg)	52 ± 8	48 ± 7
MAP (mmHg)	84 ± 8	80 ± 7
HR (bpm)	73 ± 8	68 ± 4*
Q (L/min)	6.3 ± 1.3	6.7 ± 1.5
TPR (mmHg/L/min)	14 ± 3	13 ± 3

Values are mean ± standard deviation (SD). SBP, systolic blood pressure; DSP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; Q, cardiac output; TPR, total peripheral resistance.
* Indicates $p<0.05$ for Post vs. Pre smoking cessation.

5.6 Central Vascular Mechanics

5.6.1 Systemic Vascular Stiffness -Pulse Wave Velocity

PWV to the finger was not different between pre (1426.04 ± 195.86 cm/s) and post smoking intervention (1405 ± 213.25 cm/s) (p=0.312). PWV to the toe was not different between pre (966.35 ± 147.57 cm/s) and post (953.85 ± 166.89 cm/s) (p=0.752).

5.6.2 Carotid Artery Characteristics

Carotid IMT (0.42±0.09 mm) and wall thickness (0.59±0.16 mm) post intervention were not different compared to pre intervention (IMT: 0.41 ± 0.07 mm; Wall thickness: 0.60 ± 0.14 mm) (p=0.426; p=0.325).

5.6.3 Local Carotid Mechanics

Local carotid measures of arterial stiffness and vascular characteristics are presented in Table 5.5. No significant differences occurred between groups.

Table 5.5 Local Carotid Mechanics for individuals before (Pre) and following (post) a 14 week Lifestyle Intervention Program

	Strain	Arterial Stiffness	Peterson's Elastic Modulus (mmHg)	Distensibility ($mmHg^{-1}$)	Compliance (mm/mmHg)
Pre	0.11 ± 0.03	5.57 ± 1.70	500.89 ± 139.86	$2.18 \times 10^{-3} \pm 0.72 \times 10^{-3}$	$1.30 \times 10^{-2} \pm 0.41 \times 10^{-2}$
Post	0.12 ± 0.04	5.56 ± 1.95	502.54 ± 189.12	$2.16 \times 10^{-3} \pm 0.92 \times 10^{-3}$	$1.39 \times 10^{-2} \pm 0.36 \times 10^{-2}$

Values are mean ± standard deviation (SD).

5.7 Peripheral Vascular Mechanics

5.7.1 Brachial Artery Characteristics

Brachial wall thickness was not different between pre (0.37 ± 0.08 mm) and post (0.37 ± 0.07 mm) intervention program (p=0.911).

5.7.2 Local Brachial Mechanics

Local brachial measures of arterial stiffness and vascular characteristics are given in Table 5.6. Arterial stiffness and Peterson's Elastic Modulus were decreased following smoking cessation ($p<0.05$). Compliance was not normalized to arm volume as the arm volume did not change after smoking cessation.

Table 5.6 Local Brachial Characteristics for individuals before (Pre) and following (post) a 14 week Lifestyle Intervention Program

	Strain	Arterial Stiffness	Peterson's Elastic Modulus (mmHg)	Distensibility ($mmHg^{-1}$)	Compliance (mm/mmHg)
Pre	0.03 ± 0.02	24.77 ± 16.72	2190.47 ± 1421.65	$6.43 \times 10^{-4} \pm 4.10 \times 10^{-4}$	$2.25 \times 10^{-3} \pm 1.53 \times 10^{-3}$
Post	0.04 ± 0.01	15.37 ± 5.14 *	1302.12 ± 390.73 *	$8.39 \times 10^{-4} \pm 2.60 \times 10^{-4}$	$2.83 \times 10^{-3} \pm 0.85 \times 10^{-3}$

Values are mean ± standard deviation (SD).
*Significant difference, $p<0.05$ for Post vs. Pre smoking cessation

5.7.3 Peripheral Forearm vascular properties

Forearm vascular R was decreased in post (4.33 ± 1.37 mmHg/mL/min) compared to pre (5.88 ± 2.45 mmHg/mL/min) ($p<0.01$) (Figure 5.3). Brachial MAP was decreased in post (82 ± 9 mmHg) compared to pre intervention (91 ± 11 mmHg) ($p<0.001$) (Figure 5.4). Brachial blood flow was (21.01 ± 6.93 mL/min) in quitters and similar in smokers pre intervention (19.02 ± 9.12 mL/min) ($p=0.296$) (Figure 5.5). Forearm vascular C was increased in post ($4.96 \times 10^{-3} \pm 1.99 \times 10^{-3}$ mL/min) compared to pre ($3.87 \times 10^{-3} \pm 1.96 \times 10^{-3}$ mL/min) ($p<0.05$) (Figure 5.6). Forearm vascular K remained unchanged in pre and post intervention tests (0.21 ± 0.10 mmHg/mL/min; 0.20 ± 0.07 mmHg/mL/min) ($p=0.522$) (Figure 5.7). Forearm vascular L was decreased in post ($9.81 \times 10^{-6} \pm 9.56 \times 10^{-6}$ mmHg/mL/min^2) compared to pre intervention ($3.78 \times 10^{-5} \pm 4.50 \times 10^{-5}$ mmHg/mL/min^2) ($p<0.05$) (Figure 5.8).

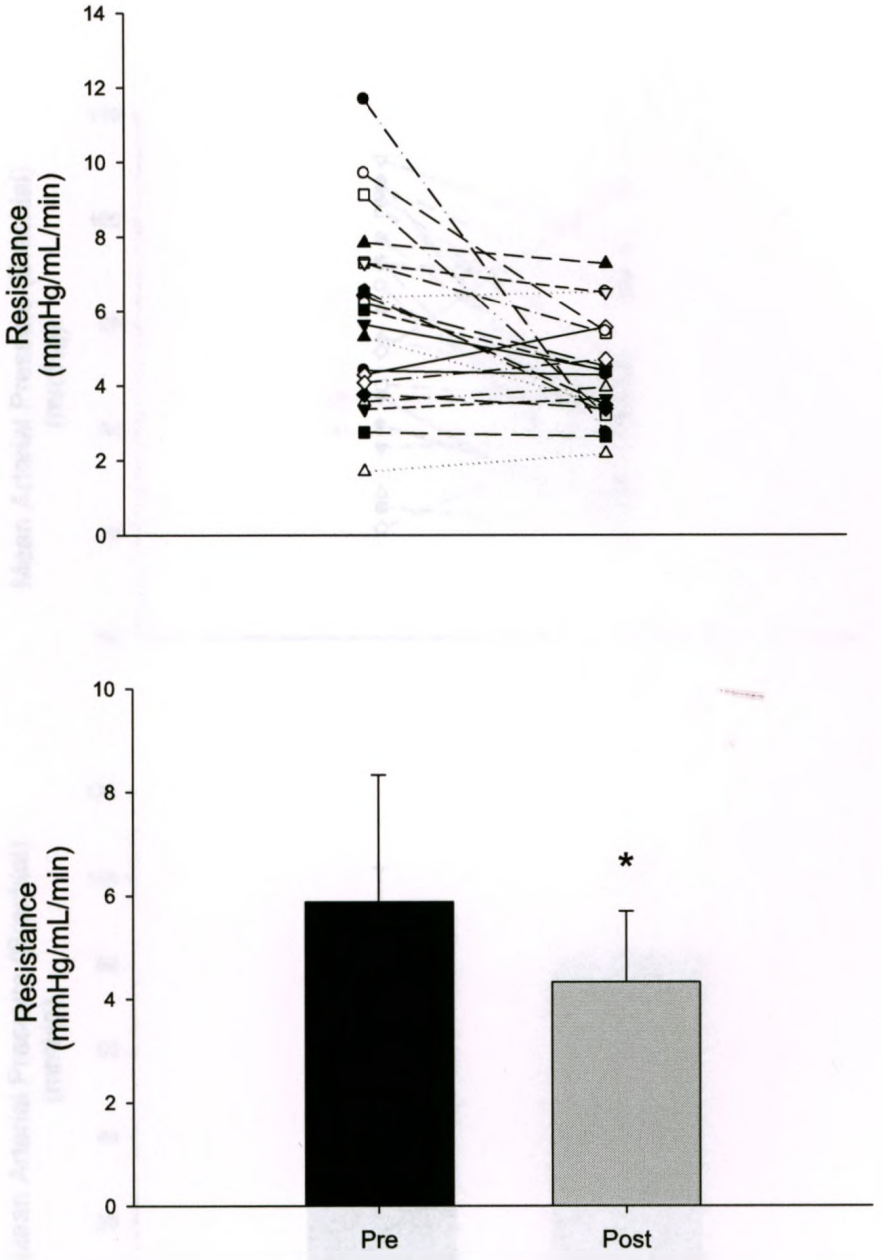


Figure 5.3 Forearm vascular resistance. Individual data (Top) and Group Mean Data (Bottom). In both graphs, Smokers, Pre lifestyle intervention are on the left (Black) and Quitters, Post Lifestyle intervention are on the right (gray). *Significant difference between groups $p<0.01$.

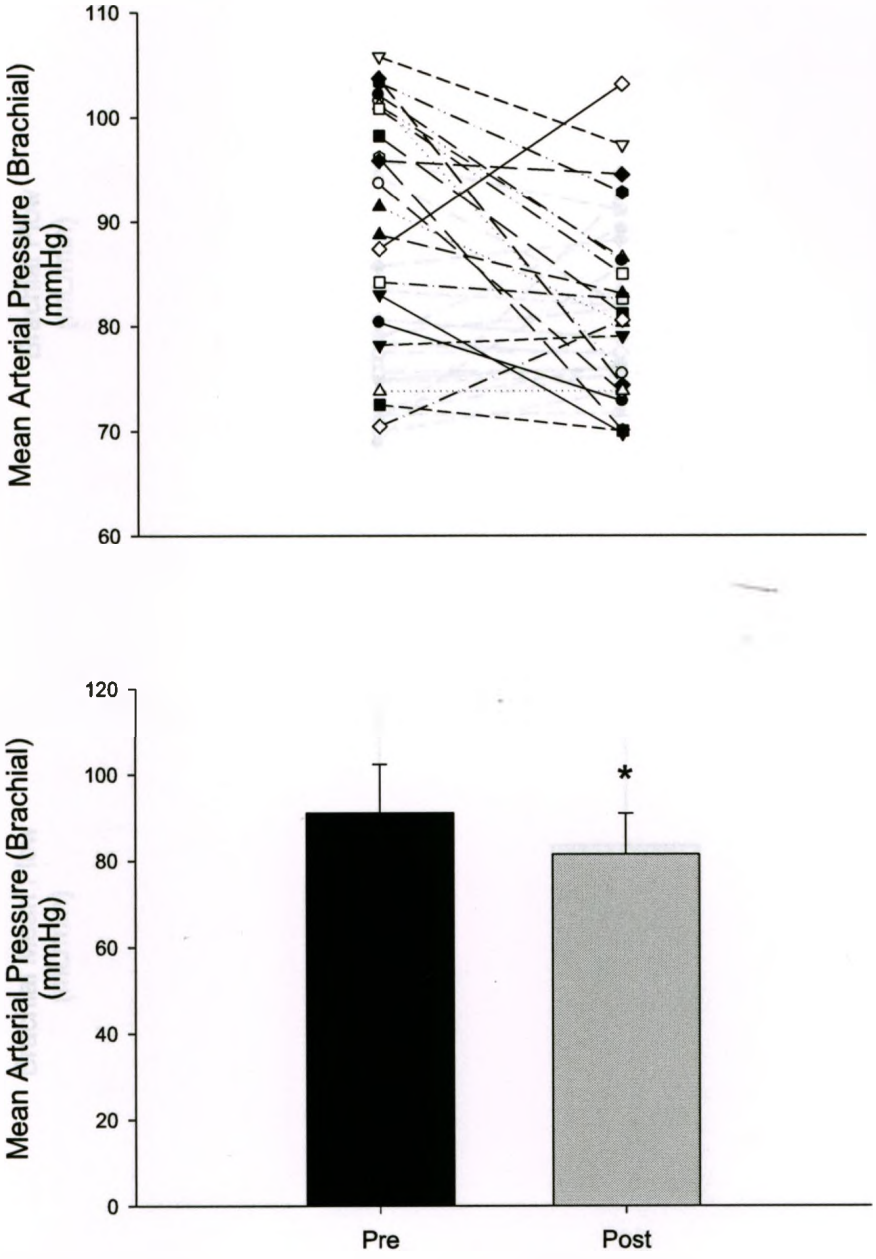


Figure 5.4 Forearm mean arterial pressure. Individual data (Top) and Group Mean Data (Bottom). In both graphs, Smokers, Pre lifestyle intervention are on the left (Black) and Quitters, Post Lifestyle intervention are on the right (gray). *Significant difference between groups $p<0.001$.

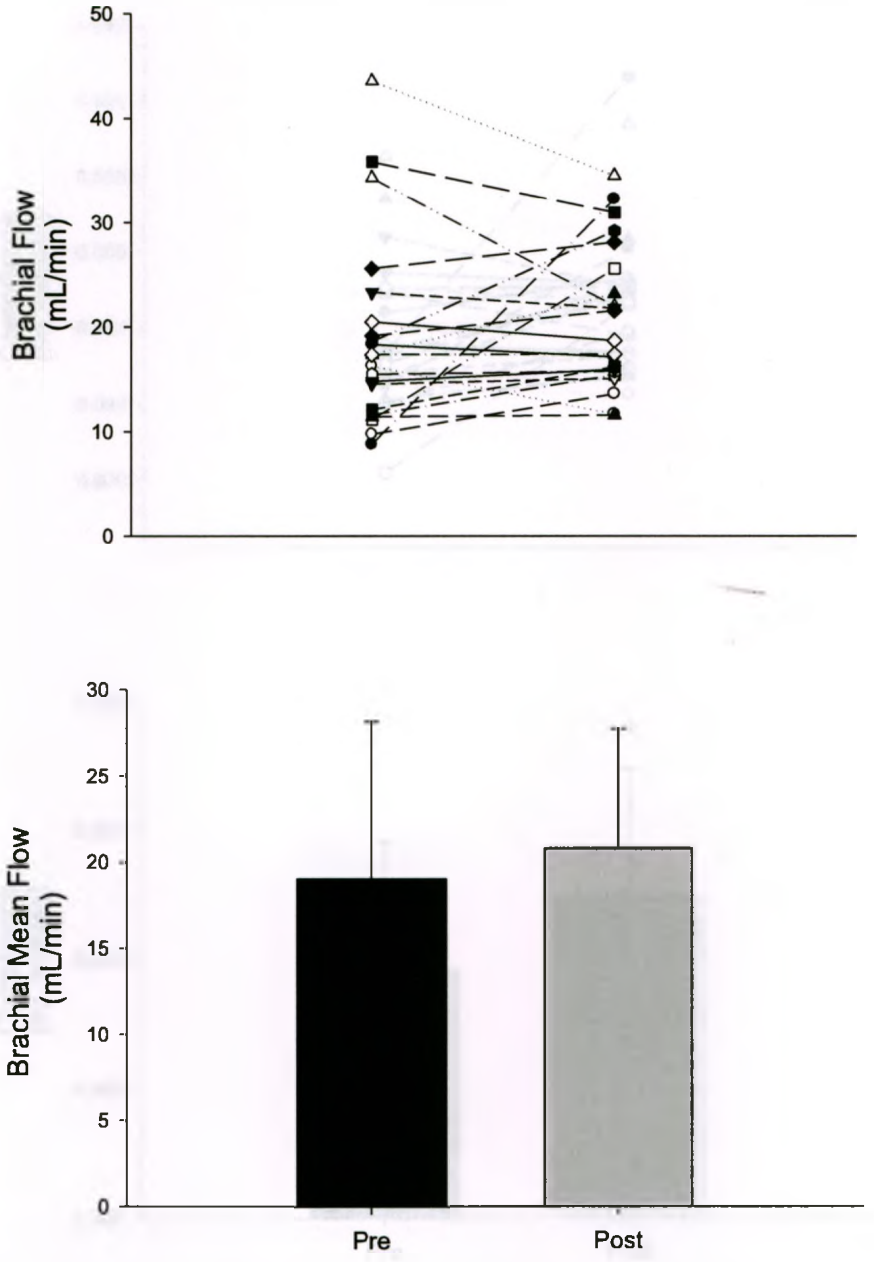


Figure 5.5 Forearm blood flow. Individual data (Top) and Group Mean Data (Bottom). In both graphs, Smokers, Pre lifestyle intervention are on the left (Black) and Quitters, Post Lifestyle intervention are on the right (gray). No significant difference between groups.

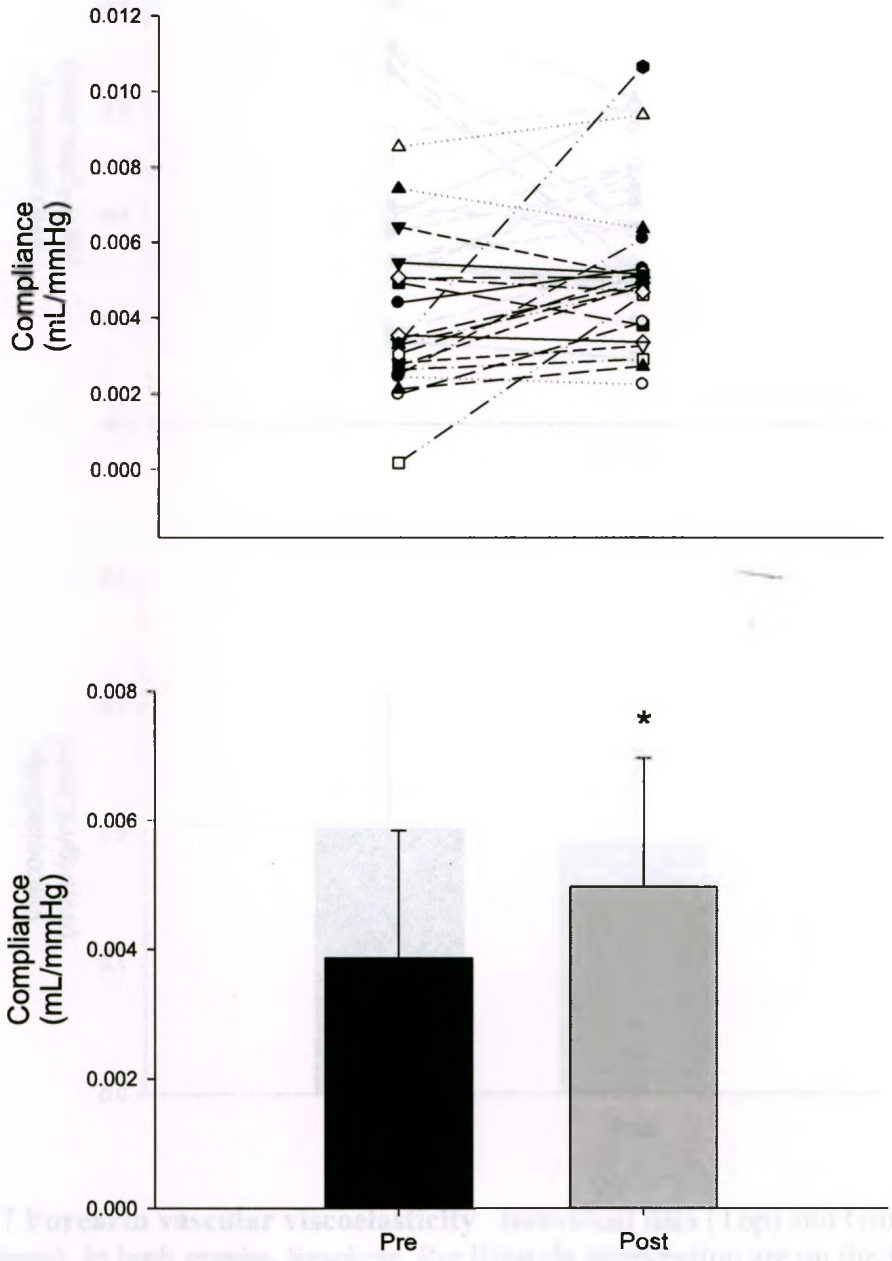


Figure 5.6 Forearm vascular compliance. Individual data (Top) and Group Mean Data (bottom). In all graphs, Smokers, Pre lifestyle intervention are on the left (Black) and Quitters, Post Lifestyle intervention are on the right (gray). *Significant difference between groups $p<0.05$.

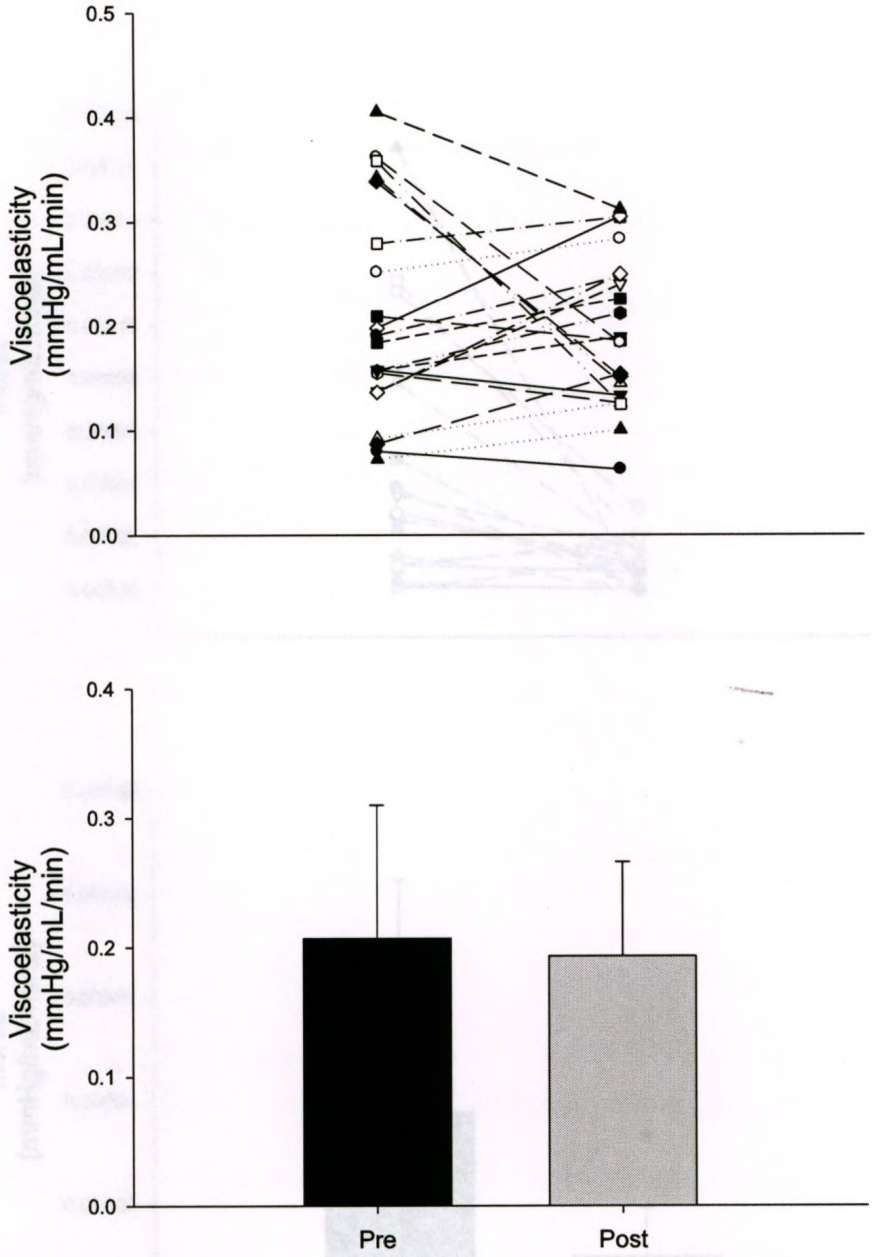


Figure 5.7 Forearm vascular viscoelasticity. Individual data (Top) and Group Mean Data (Bottom). In both graphs, Smokers, Pre lifestyle intervention are on the left (Black) and Quitters, Post Lifestyle intervention are on the right (gray). No significant difference between groups.

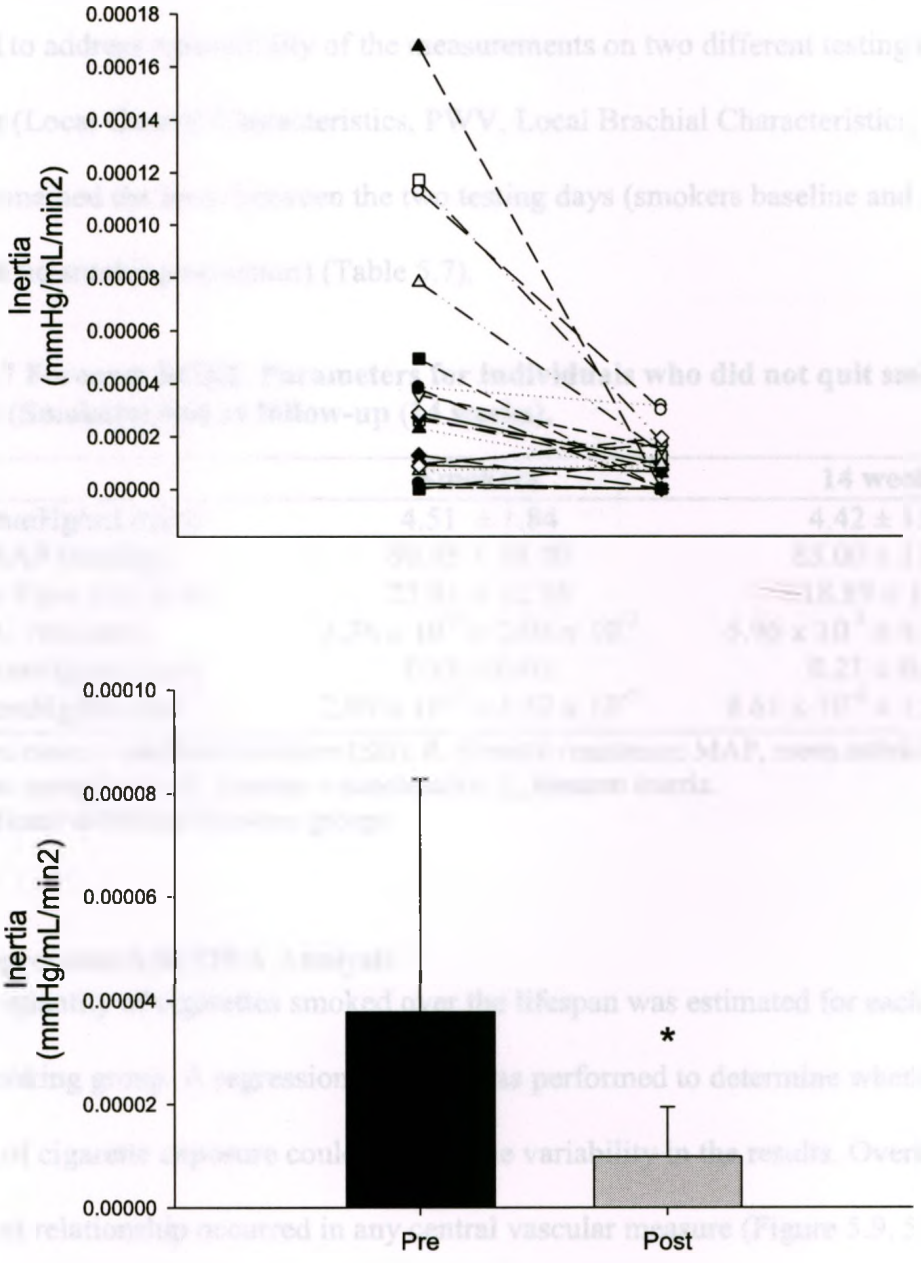


Figure 5.8 Forearm vascular inertia. Individual data (Top) and Group Mean Data (Bottom). In both graphs, Smokers, Pre lifestyle intervention are on the left (Black) and Quitters, Post Lifestyle intervention are on the right (gray). *Significant difference between groups $p<0.05$.

5.8 Sub Analysis

5.8.1 Internal Controls

Seven participants who did not quit smoking but returned for follow-up testing were analyzed to address repeatability of the measurements on two different testing days. All variables (Local Carotid Characteristics, PWV, Local Brachial Characteristics, R, C, K, and L) remained the same between the two testing days (smokers baseline and 14 weeks later with no smoking cessation) (Table 5.7).

Table 5.7 Forearm RCKL Parameters for individuals who did not quit smoking at baseline (Smokers) and at follow-up (14 weeks).

	Smokers	14 weeks
R (mmHg/mL/min)	4.51 ± 1.84	4.42 ± 1.02
MAP (mmHg)	90.85 ± 14.70	83.00 ± 17.23
Mean Flow (mL/min)	23.91 ± 12.33	18.89 ± 1.61
C (mL/min)	$3.76 \times 10^{-3} \pm 2.03 \times 10^{-3}$	$5.96 \times 10^{-3} \pm 4.18 \times 10^{-3}$
K (mmHg/mL/min)	0.18 ± 0.01	0.21 ± 0.05
L (mmHg/mL/min^2)	$2.09 \times 10^{-5} \pm 1.52 \times 10^{-5}$	$8.61 \times 10^{-6} \pm 1.52 \times 10^{-5}$

Values are mean ± standard deviation (SD). R, forearm resistance; MAP, mean arterial pressure; C, forearm compliance; K, forearm viscoelastcity; L, forearm inertia.
No Significant difference between groups

5.8.2 Regression/ANCOVA Analysis

The quantity of cigarettes smoked over the lifespan was estimated for each individual in the smoking group. A regression analysis was performed to determine whether the quantity of cigarette exposure could address the variability in the results. Overall, no significant relationship occurred in any central vascular measure (Figure 5.9, 5.10) nor peripheral vascular measure (Figure 5.11, 5.12).

Groups were age-matched prior to data collection and an analysis of covariance verified that age did not influence the differences that were observed ($p>0.05$). In addition differences between groups were not influenced by day of menstrual cycle ($p>0.05$).

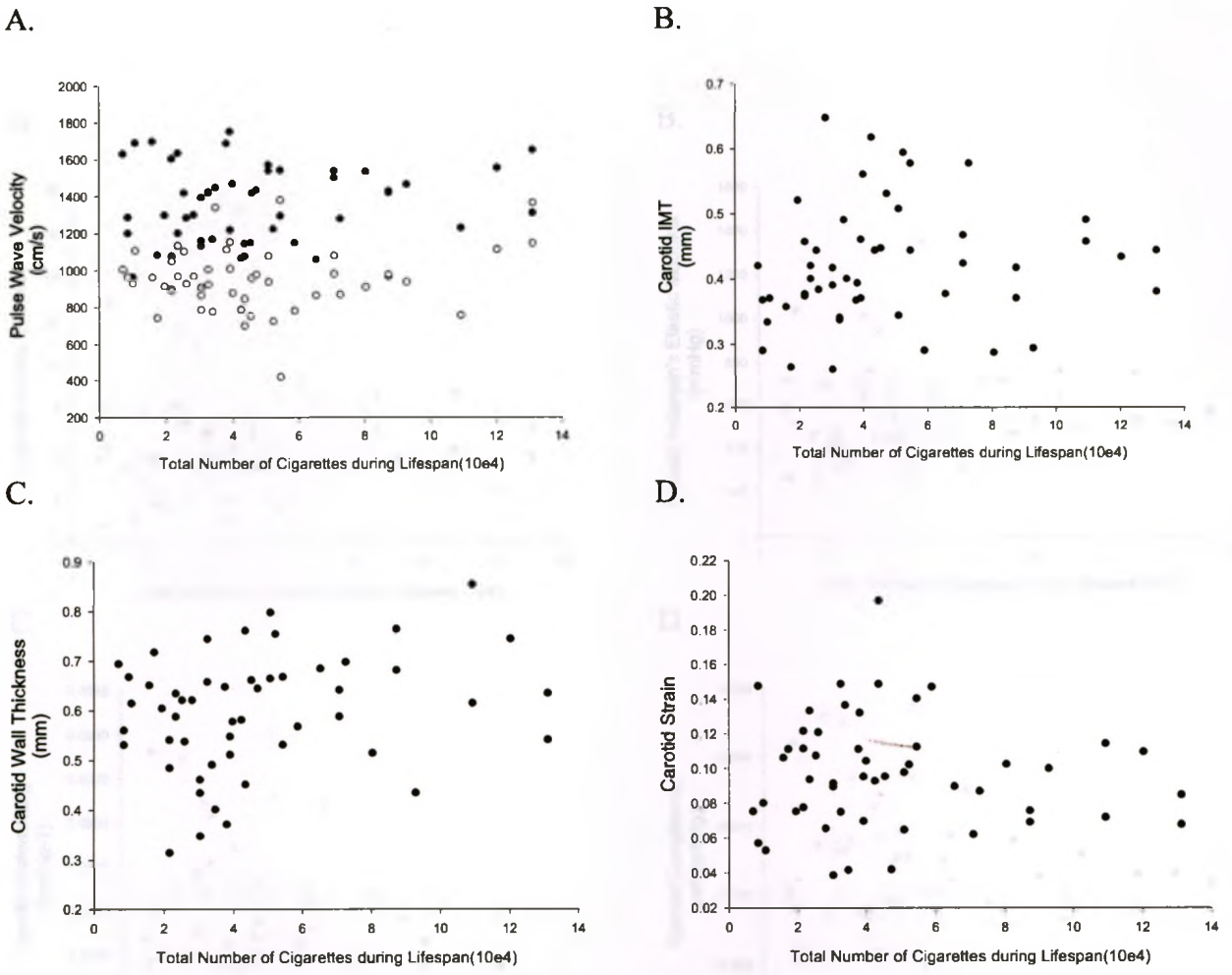


Figure 5.9 Regression Analysis of Smoking Quantity vs. Central Mechanics. A. Pulse Wave Velocity to the Finger (black) and toe (white); B. Carotid IMT; C. Carotid Wall Thickness; D. Carotid Strain; All variables are plotted against the estimated total number of cigarettes smoked during the subject's lifespan. No relationship occurred for any parameter.

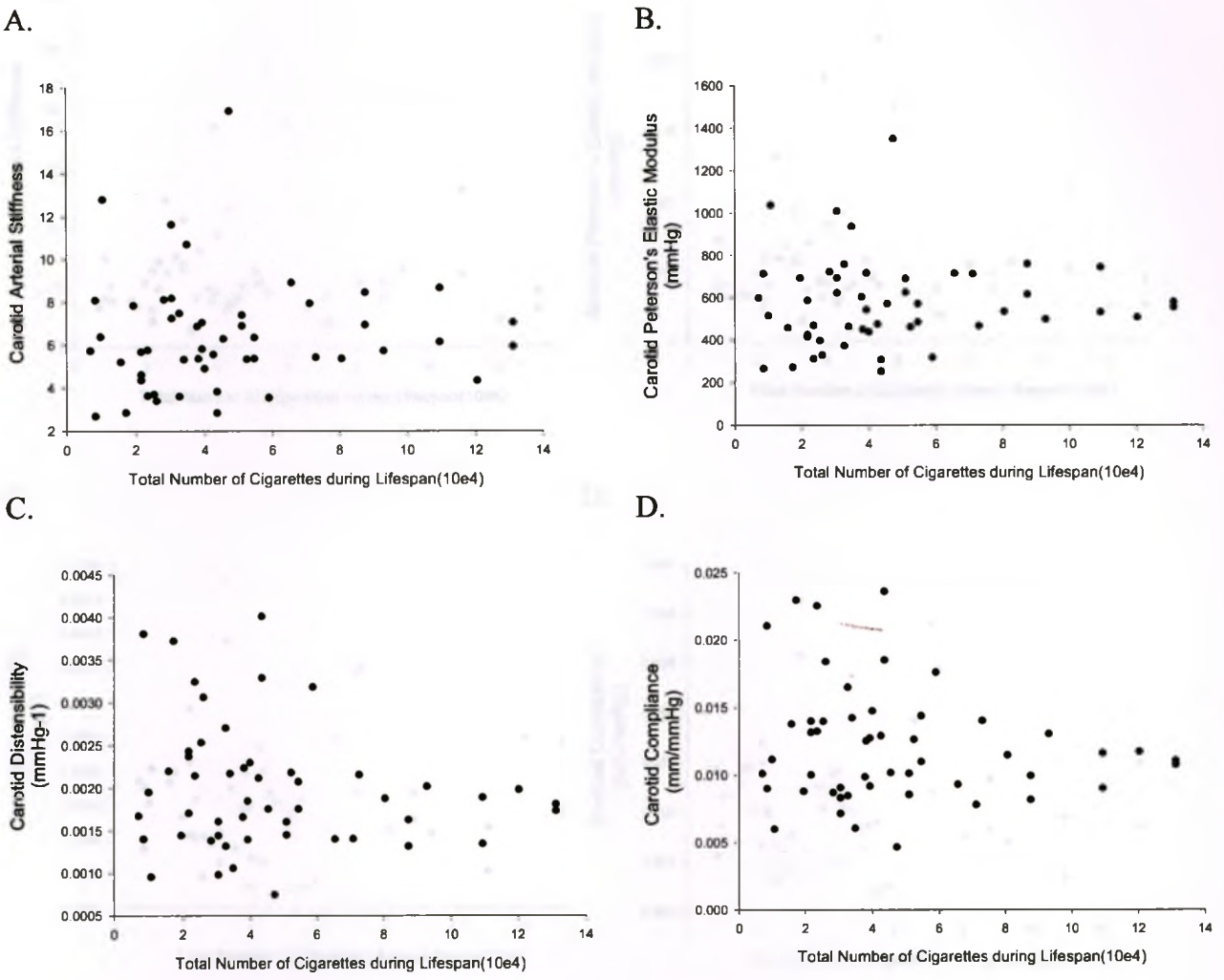


Figure 5.10 Regression Analysis of Smoking Quantity vs. Central Mechanics. A. Carotid Arterial Stiffness; B. Carotid Peterson's Elastic Modulus; C. Carotid Distensibility; D. Carotid Compliance. All variables are plotted against the estimated total number of cigarettes smoked during the subject's lifespan. No relationship occurred for any parameter.

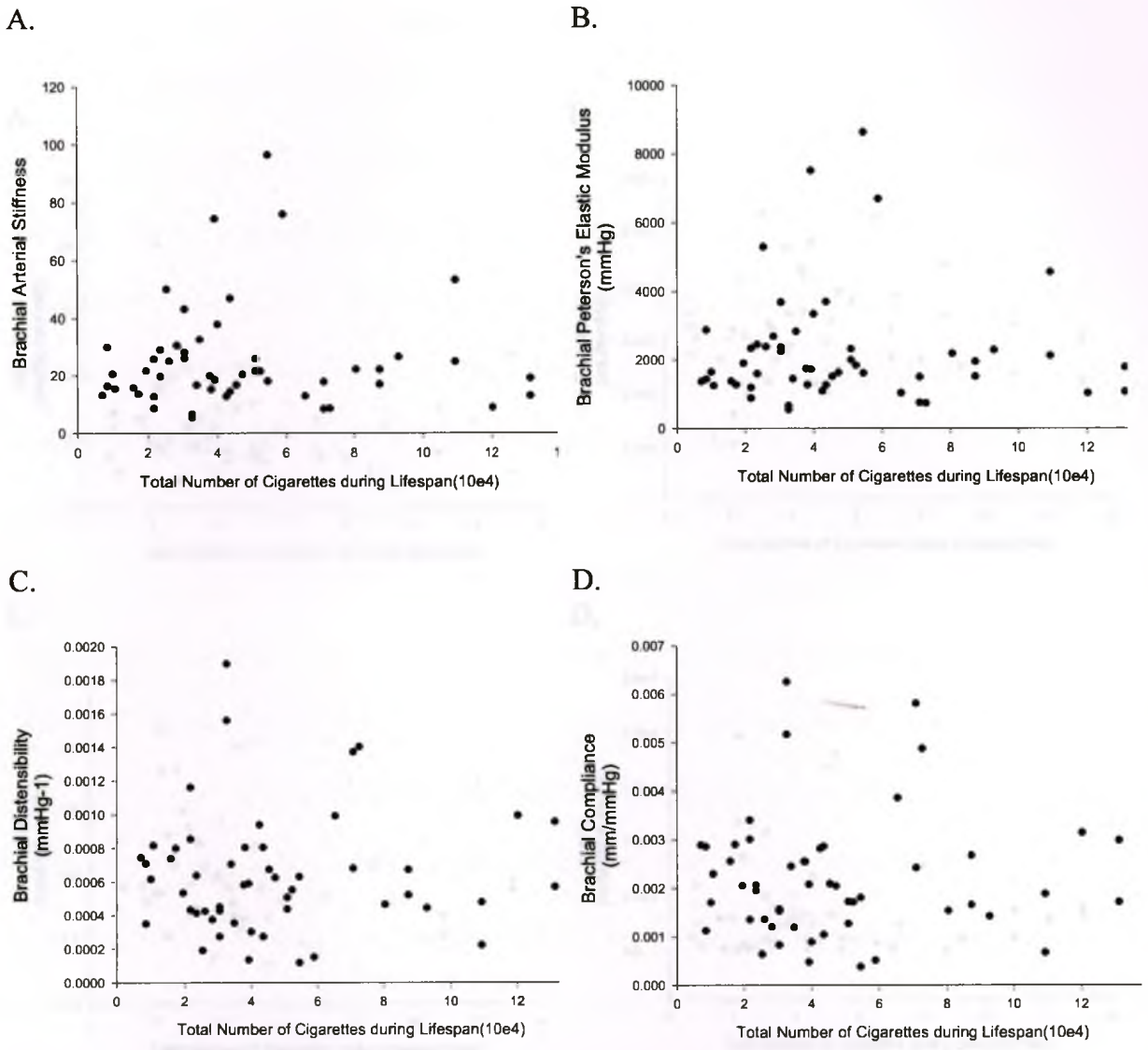


Figure 5.11 Regression Analysis of Smoking Quantity vs. Peripheral Local Mechanics. A. Brachial Arterial Stiffness; B. Brachial Elastic Modulus; C. Brachial Distensibility; D. Brachial Compliance. All variables are plotted against the estimated total number of cigarettes smoked during the subject's lifespan. No relationship occurred for any parameter.

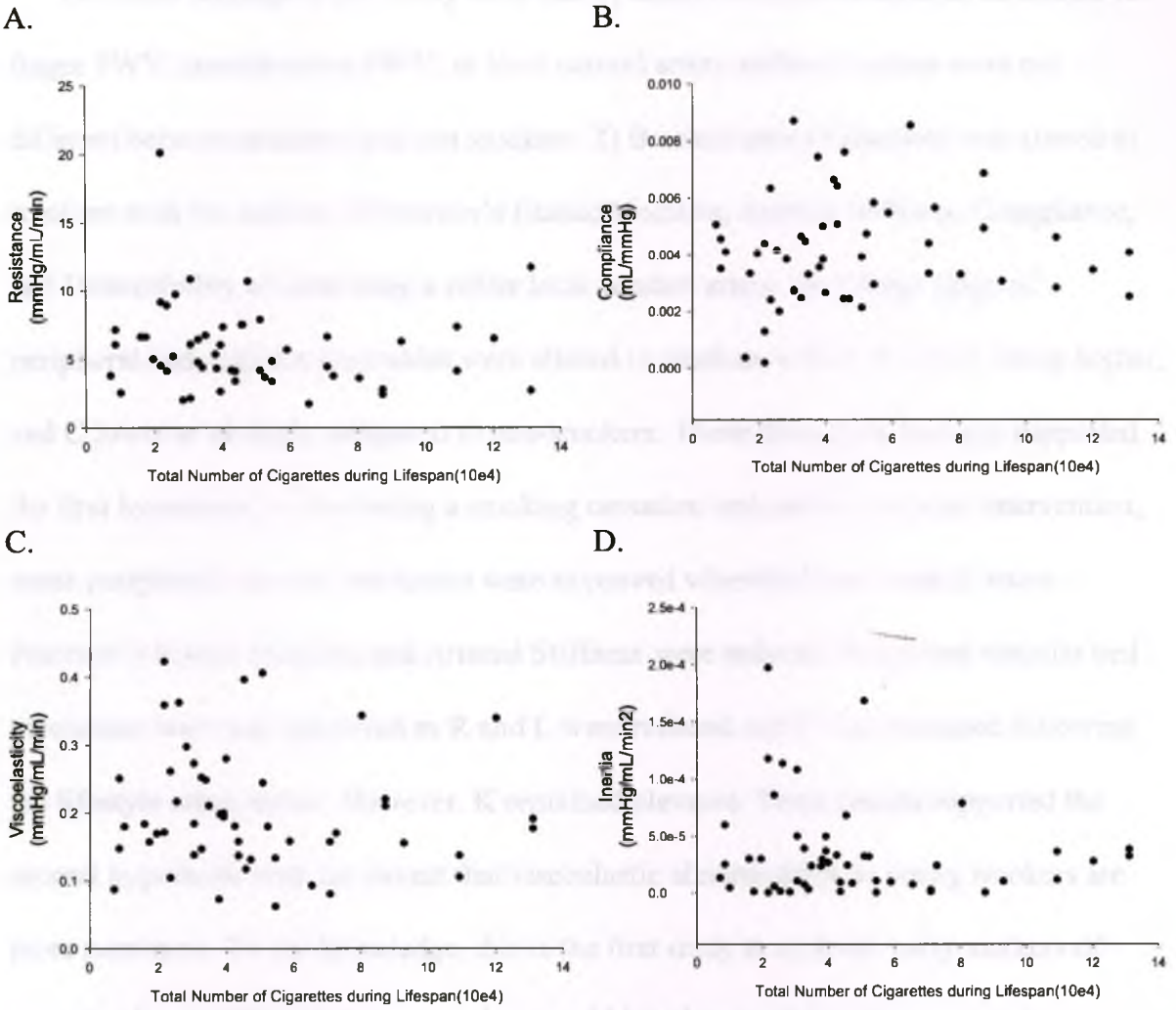


Figure 5.12 Regression Analysis of Smoking Quantity vs. Peripheral Mechanics. Determined from the RCKL Model. A. Resistance; B. Compliance; C. Viscoelasticity; D. Inertia. All variables are plotted against the estimated total number of cigarettes smoked during the subject's lifespan. No relationship occurred for any parameter.

CHAPTER 6 : DISCUSSION

The main findings of this study were that 1) central vascular mechanics of carotid-to-finger PWV, carotid-to-toe PWV, or local carotid artery stiffness indices were not different between smokers and non smokers. 2) Brachial artery behaviour was altered in smokers with the indices of Peterson's Elastic Modulus, Arterial Stiffness, Compliance, and Distensibility all indicating a stiffer local conduit artery. 3) A large range of peripheral bed vascular mechanics were altered in smokers with R, K, and L being higher, and C lower in smokers compared to non-smokers. These three main findings supported the first hypothesis. 4) Following a smoking cessation and aerobic exercise intervention, some peripheral vascular mechanics were improved whereby local brachial artery Peterson's Elastic Modulus and Arterial Stiffness were reduced. Peripheral vascular bed mechanics were also improved as R and L were reduced and C was increased following the lifestyle intervention. However, K remained elevated. These results supported the second hypothesis with the caveat that viscoelastic abnormalities in young smokers are more persistent. To our knowledge, this is the first study to a) detect early markers of vascular dysregulation in young smokers, and b) to demonstrate improvements in vascular mechanics in a little as 14 weeks following a lifestyle intervention.

6.1 Hemodynamic Variables

All participants in this study were deemed healthy and did not exhibit any signs of cardiovascular disease or hypertension. HR, PP, TPR, Q, and SV were the same between both groups. Although within the clinically safe definition, SBP, DBP, and MAP were elevated in the smokers compared with non-smoker controls. The latter observations are supported by other studies that also demonstrate increased BP in smokers (95; 97; 112). Also, the BP was reduced in the smoking cessation group to values that were similar to

the non-smoking control group. These results suggest that the smoking behaviour initiated an increased BP in smokers compared to non-smokers. The mechanism of the increased systemic BPs in smokers is not certain but the decreased compliance of the peripheral vascular bed in smokers (Discussed in Section 6.2.3), may contribute due to the increased wave speed of the reflected wave causing the forward and reflected wave to augment SBP (93; 109). However, PP, which reflects the dynamic components of the arterial vasculature, was not different between the two groups encouraging care be taken in ascribing a central impact of peripheral vascular stiffening.

6.2 Central Vascular Mechanics

In the current study, central vascular mechanics were assessed first by PWV across the entire vascular bed. This approach likely produces a weighted average of PWV across the various vascular segments consisting of the highly elastic aorta to the muscular femoral artery, to the microvessels. Systemic measurement of arterial stiffness, as indicated by PWV, was not different between smokers and non-smokers. This is supported by numerous studies who also found no difference in PWV in smokers (95; 97; 112; 156). However, other studies have demonstrated an increase in PWV in smokers (87; 190; 204). The reasons for the discrepancy remain obscure. However, one factor may be the timing of the measures within the smoking behaviour. For example, consistency in PWV change is reported in studies investigating the effect of smoking one cigarette. Acutely, smoking causes increased PWV and systemic stiffening (54; 95; 97; 98; 204). Also, the chronic increase in PWV may result from greater smoking years and age-related changes (87; 204).

Second, discrete measurements of carotid artery vascular stiffness were included as markers of local central artery changes to represent characteristics of central elastic arteries in the absence of other segments. Alterations in these features of the carotid artery are highly correlated to the presence and likelihood of developing severe atherosclerosis or vascular disease (178; 203). However, as with systemic PWV, no differences were observed between smokers and non-smokers in any measure of carotid artery vascular mechanics.

Our values of carotid artery mechanics are consistent with normative data of healthy young subjects as reported in a review of current literature by O'Rourke *et al.* (2002) (145). Importantly, the current results support previous findings in young smokers where no change in large artery compliance or distensibility was found (195). In addition, smokers in the current study exhibited no increase in IMT and, therefore, showed no signs of atherosclerotic plaque in the carotid artery.

Other studies investigating the effects of smoking in older individuals have found increased local stiffness (87) and IMT (9; 13; 150) suggesting that smokers will exhibit alterations in central arteries. The discrepancy in findings may be accounted for by the age of the participants. In the majority of studies the age range is wide and the average age is greater than 40 years of age (9; 10; 87). The observed differences between the current and previous reports may also be accounted for by the age of the participants who may have been smoking for a greater number of years (191). Thus, central vascular alterations may occur later in life due to the progression of vascular disease from the peripheral to central arteries. This progression may be accelerated once an individual

reaches a certain age and presents other confounding factors such as high BP, high cholesterol, and obesity (94; 205).

6.3 Peripheral Vascular Mechanics

To our knowledge, this study is the first to describe peripheral vascular mechanics in young smokers. This new evidence was due to the inclusion of local brachial artery and forearm vascular bed mechanics in the analysis, both of which point to a stiffer and remodelled forearm vascular bed.

The peripheral muscular brachial artery demonstrated alterations of local vascular mechanics. Arterial stiffness was increased in smokers and was demonstrated by elevated Peterson's Elastic Modulus and decreased compliance and distensibility of the brachial artery. The elevation in Peterson's Elastic Modulus demonstrates the increased pressure required to inflate the artery. A reduced compliance and distensibility represents a decreased distension for a given pressure. Despite these results, the majority of studies have not found any changes in muscular artery stiffness (97; 195). However, as outlined above, the age ranges of these previous studies were different and these studies grouped male and females together. Various studies have demonstrated large differences between males and female subjects (31; 58) and therefore grouping both sexes together may wash out any differences in smokers. The current analysis studied exclusively the vascular properties of female smokers, providing a more homogeneous population but also forming a distinct delineation for interpreting the results.

Incorporating a Windkessel modelling approach for the forearm vascular bed identified peripheral bed vascular mechanics and was able to detect changes in smokers compared to non-smokers. This is a lumped model in that it incorporates all levels of the

vascular bed below the point of measurement. Thus, there is a proportionately larger element of microvascular contribution to these data than any other method of studying vascular health noninvasively in humans. McVeigh *et al.* (1997) were the first to observe altered peripheral vascular mechanics in smokers in the absence of changes to central vascular properties (122). The decrease in peripheral compliance observed was identified by pulse contour analysis and therefore may pose some limitations (122).

Our results also demonstrate altered peripheral vascular mechanics as R was elevated in smokers due to an increased MAP. R reflects the steady state of blood flow and the pressure that the heart must generate to deliver adequate blood to the periphery. This increased R in smokers will increase the load on the heart and may result in left ventricular remodelling over time (93; 109). Increased R, controlled by the diameter of the downstream vasculature, identified a more constricted system in smokers which may be due to increased sympathetic activity (24; 135; 198) and/or decreased dilatory compounds such as nitric oxide (147; 152) and sex hormones (19; 75; 123) in smokers.

The dynamic parameters regulating oscillatory blood flow, namely C, K, and L, were also altered in smokers. Decreased C and increased K demonstrate the reduced capability of the vessel wall to stretch and store blood volume during systole so that the vascular recoil can contribute to continuous blood flow during diastole. L, the energy required to accelerate the blood from the heart, was also elevated in the young smokers.

In support of our findings, Messerli (126) observed a greater increase in arterial stiffness compared to the increase in BP. Although this study was conducted in hypertensive patients, it was suggested that the increased stiffness was also due to

structural remodelling. Avolio *et al.* (1998) linked the structural remodelling and stiffening to the load bearing properties of collagen, which may also relate to the resistance to stretch of the vessel wall, and contribute to increased K and decreased C (11). The degradation of elastin may also contribute to the decreased compliance (41; 207).

However, the C and K of the vessel wall are not regulated by structural components alone. In diseased vessels, the myogenic response may be altered as increased cross-linking between actin and myosin and increased calcium levels may increase the contraction response to stretch (11; 41). This may decrease the compliance and increase the viscoelasticity of the vessel wall. The inability to dilate is also impaired in both smokers and in a diseased vessel, as the reduction in endothelial function (104; 121; 204; 213) may reduce nitric oxide and other vasodilatory molecule formation and contribute to decreased arterial compliance. In addition, increased sympathetic activity (24; 135; 198) and decreased estrogen (19; 75; 123) and signalling molecules (147; 152) observed in smokers are other mechanisms that may increase the resistance and visoelasticity and decrease the compliance of the vessel wall. Although these were not measured in this study, they may provide mechanisms that alter the parameters controlling blood flow.

In the current study we have demonstrated the limitation of focusing solely on central artery changes when searching for early markers of vascular maladaptations. Central vascular mechanics and/or systemic stiffness are the major outcome variables for the majority of clinical and research studies investigating risk factors and the progression of vascular disease. The findings of this study demonstrate the importance of studying peripheral vascular bed mechanics.

6.4 Effect of a Lifestyle Intervention on Peripheral Vascular Mechanics

Following a lifestyle intervention of aerobic exercise and smoking cessation, peripheral vascular mechanics were improved. While local brachial artery compliance and distensibility were not improved, local Peterson's Elastic Modulus and Arterial stiffness were decreased following the lifestyle interventions. Furthermore, forearm vascular bed mechanics also demonstrated improvements following the lifestyle intervention. Specifically, R was decreased and returned the quitters to similar levels as non-smokers. Importantly, this reduction in R appeared to be due to a decreased MAP as forearm blood flow remained constant. However, the change in R suggests an overall dilation of the forearm vascular bed related perhaps to a myogenic mechanism. Although forearm C was improved post intervention, C was still decreased compared to non-smokers. This suggests that C is a malleable feature of the forearm and that, perhaps, additional time is required for full restoration of this variable. The improvement in C supports previous studies that observed an increase in arterial compliance following aerobic exercise (61; 185) and a decreased PWV following smoking cessation (87) and thus decreased stiffness. In contrast, forearm K remained unchanged between the pre and post intervention measurements and persistently higher compared to non-smokers. Thus, the viscoelasticity of the forearm bed demonstrates resistance to change, relative to the other mechanical properties. Importantly, this differential response suggests that K can be independent from C or L, as suggested earlier (210-212). Similarly, vascular bed L was improved following the intervention and was lowered similar to non-smokers. Vascular bed L is probably one of the least understood parameters regulating blood flow. However, the improvement of forearm L represents an improved matching of blood flow

and pressure. The lower the inertia, the decreased time it will take the flow to reach steady state (36; 210).

This study is the first to our knowledge to demonstrate improvements in peripheral vascular mechanics in as little as 14 weeks following aerobic exercise and smoking cessation. However, the incomplete recovery of these properties points to the need for longer-term interventions to determine whether complete reversal can be achieved.

It may be of interest to determine whether the differential impact of lifestyle modification on peripheral versus central vascular properties are specific to smokers or if this is a general pattern of vascular dysregulation. In this context, the current findings are in agreement with the study by Aizawa *et al.* (2009) who found that an eight week aerobic exercise program significantly increased brachial arterial compliance but not central arterial compliance in older adults with metabolic syndrome (3). This earlier study was not able to assess the forearm vascular bed. Nonetheless, the sustained increase in K and lower C post intervention in smokers of the current study, compared to non-smokers, may allude to the persistence of smoking-induced alterations. Increased collagen, smooth muscle cell proliferation, and decreased elastin may contribute to these altered parameters. The purpose of this study was not to determine the mechanisms for alterations in these parameters, but the pathophysiology regulating these parameters will be important to study in the future.

The improvement in compliance may also play a role in the reduced MAP which then decreased R. An improved C in the peripheral bed should decrease the wave speed of the reflected wave and may decrease the augmentation of systolic pressure seen pre

intervention in smokers (137). Arterial compliance is represented as the slope at a specific point on the pressure-volume curve (102). The pressure-volume curve would indicate that the artery is more compliant at low BP and less compliant at high BP. In the current study, both SBP and DBP were significantly reduced following the intervention, causing a leftward shift in the pressure-volume curve. It is possible that this reduction in BP contributed to the observed improvement of peripheral arterial C. However, similar to Cameron *et al.* (1994) the increase in compliance (28%) is greater than that due to changes in BP (5%) and is likely to include a component due to change in intrinsic arterial compliance (39). Our results support the proposition that there is a primary, lifestyle intervention-induced, change in vascular compliance in excess of that explained by an associated fall in BP.

We can only speculate on the mechanism by which the intervention program improved peripheral vascular mechanics in the present study. Arterial compliance is partly determined by the intrinsic elastic properties of the artery, namely the composition of elastin and collagen (structural determinants) and the vasoconstrictor effect of smooth muscle cells (functional determinant) (137; 179). In as little as 3 months of exercise training, Dinenno *et al.* (2001) found that significant arterial remodelling occurs in the femoral artery (48). It is also possible that the increased pressure and mechanical distension during exercise sessions "stretched" collagen fibres and modified their cross-linking, thereby increasing arterial compliance (35). In addition, tension generated by the connective tissue is reduced in a dilated vessel (179).

Arterial compliance can be altered over a short time period, even acutely, via modulation of the sympathetic adrenergic tone of smooth muscle cells in the arterial wall

(17; 33; 211). Also, a reduction of nicotine, which has been observed to increase smooth muscle cell proliferation (40), may also contribute to decreased stiffness and constriction regulated by smooth muscle cells. In this context, it is possible that regular aerobic exercise and smoking cessation, both which may decrease sympathetic activity (125; 129; 132; 146; 160; 209), combined with an elevated sympathetic activity due to cigarette smoking pre intervention (135), reduced the chronic sympathetic activity post intervention. Exercise training also increases shear stress and endothelial function. Although smokers demonstrate reduced nitric oxide bioavailability (147), and thus reduced endothelial function (156; 213), the ability of smokers to dilate in response to exogenous vasodilatory sources is intact (121; 204) and may provide evidence for the short term improvements in peripheral vascular function. Following smoking cessation, nicotine and other compounds in cigarettes are no longer present to reduce nitric oxide and other vasodilatory compounds availability. In fact, following smoking cessation evidence suggests that nitric oxide concentration is restored compared to non-smokers (141). Therefore, the endothelial dysfunction in smokers may be restored as increased levels of vasodilatory molecules are present. Future studies will be needed to determine whether sympathetic activity is reduced and endothelial function restored following this type of combined intervention.

In summary, the results support the hypothesis that peripheral parameters can be improved without changes in central parameters. Since the smokers were young and showed no signs of cardiovascular disease, it was not expected that central vascular mechanics would be significantly altered compared to non-smokers. In those smokers that

were not successful in smoking cessation, the likelihood of central vascular alterations in their lifespan is higher.

6.5 Correlation Analysis

To address the discrepancy from previous studies that suggest that the number of smoking years is (10; 51; 133; 148; 149) or is not (13; 21), correlated to cardiovascular risk factors, we also investigated possible correlations in peripheral and central vascular mechanics. Overall, this study demonstrated no association between any central or peripheral variable and the quantity of cigarettes smoked. Also, the vascular indices were not related to the participant's age. Although not unexpected, due to support from previous studies, these results were surprising in that we studied both central and peripheral factors and demonstrated smoking induced alterations. Although our participants were young we still were able to include a wide range of smoking years, ranging from 2 to 23 years smoking. A possible explanation for the lack of correlation may be that the altered peripheral vascular mechanics are due to properties regulating blood flow that are turned 'on or off' with smoking. For example, cigarette smoke causes an increase in sympathetic activity (24; 135; 198) which may cause constriction, increased R, and/or decreased C. Thus, while each cigarette may acutely cause neurogenic vascular stiffening, the continuous smoking results in a chronic sympathetic activation with continuous changes in peripheral vascular stiffness. Certainly, recent evidence points to the stiffening impact of reflex sympathetic activation on the brachial artery (33; 165). Alternatively, structural remodelling in the periphery may take significantly less time than central remodelling, as supported by the little time required to improve these characteristics following our lifestyle intervention. In addition, Weil *et al.*

(2009) observed endothelial dysfunction in young smokers who had been smoking for less than 5 years (199). Support for these hypotheses requires future testing.

6.6 Implications

This study has clinical and public health implications. The clinical relevance of our findings lies in our ability to detect alterations in peripheral vascular mechanics in the absence of central changes in young smokers. The importance of detecting early changes in vascular stiffness indices is that these may reflect the early onset of vascular disease. Our study also demonstrated that a 14-week aerobic exercise and smoking cessation intervention produced a significant reduction in peripheral microvasculature arterial stiffness indices in young women who smoke. Therefore, this intervention reduced the risk level for cardiovascular pathology in these women and may prevent cardiovascular diseases later in life. It is important to note as well that without measures of peripheral vascular bed properties, the benefit of the smoking cessation program would not have been observed. The improvement of arterial stiffness is an important outcome for the primary prevention of cardiovascular disease and demonstrates the need to identify successful intervention strategies to implement in public health. Our study demonstrated that a laboratory-based intervention of smoking cessation and aerobic exercise is an effective means to achieve these positive results.

6.7 Limitations

The Windkessel model used to study the forearm vascular bed is a lumped model and is not able to distinguish the R, C, K, or L of single vessels or segment levels of the peripheral arterial tree.

We are also limited in our ability to conclude whether the aerobic exercise or smoking cessation intervention caused the improved peripheral vascular mechanics. Additional studies are required to determine the independent effects of aerobic training or smoking cessation in this population. However, the purpose of this study was not to evaluate whether one or the other could result in improvements; rather, it was to evaluate whether these vascular alterations could be modified at all by a comprehensive lifestyle intervention.

For logistical reasons of scheduling tests and training, this study did not standardize the phase of menstrual cycle for participants. However, in our sub-analysis we observed no relationship to central or peripheral vascular mechanics relative to the day of cycle. Therefore, the impact of smoking and the intervention in this study were not related to menstrual phase during testing.

Finally, carotid arterial compliance and distensibility calculations used PP values from the brachial artery since we could not measure BP at the carotid artery. While numerous studies employ the use of this technique (2; 2; 97; 195), this may have introduced an error in these calculations as brachial BP may overestimate carotid BP (137). However, in anesthetized dogs, a linear relationship was found between pressure in the brachial artery and pressure in the common carotid artery over a relatively wide range of pressures (158).

CHAPTER 7 : CONCLUSION

The results of the present study extend the prior findings of the effects of smoking on central and peripheral vascular mechanics.

First, our cross sectional study allowed us to determine the effect of smoking on both central and peripheral vascular mechanics. To our knowledge, this is the first report of segmental vascular mechanics in at-risk individuals. We found that reductions in central artery stiffness were not present in young women smokers. However, peripheral vascular mechanics were altered in smokers as R, K, and L were all elevated compared to non-smokers. These variables have not been reported previously in this population. Further, vascular compliance of the brachial artery locally, and of the entire forearm vascular bed (C) using the windkessel model, were lower in smokers.

Second, the follow-up study demonstrated that a 14 week lifestyle program of aerobic exercise and smoking cessation can improve peripheral vascular compliance in women who smoked previously. Moreover, this allowed us to examine the parameters regulating blood flow in the periphery identified as R, C, K, and L. These results suggest that a relatively short intervention of aerobic exercise and smoking cessation in healthy young women smokers can restore some of the dynamic arterial parameters regulating blood flow. However, the sustained increase in K demonstrates the persistence of this effect of smoking.

Our results have a number of potentially important clinical implications. Reductions in arterial compliance are believed to contribute significantly to the pathophysiology of vascular and cardiac diseases as cardiac function is affected by increasing the aortic

impedance to ejection of left ventricular SV (109). This, in turn, may contribute to reductions in left ventricular performance. In addition, vascular stiffening precedes atherosclerotic plaque build-up, increasing the likelihood of a heart attack or stroke (6). In this study, there was no difference in central vascular stiffness between young smokers and non-smokers suggesting that the vascular changes that are observed with smoking develop first in the periphery and move centrally. Thus, the decreased peripheral arterial C, increased R, K, and L may provide the first evidence of earlier vascular disease markers in young smokers. Our results also demonstrate that despite the negative alterations in peripheral vascular mechanics in young smokers, some of these parameters can be improved in as little as 14 weeks of a lifestyle intervention. The early detection of vascular disease may significantly impact intervention strategies and decrease one's increased risk of developing vascular disease. Delineation of the mechanisms to explain these findings was not the purpose of this study and remain to be elucidated.

In perspective, this study illustrates the importance of assessing vascular mechanics in the peripheral vascular bed as well as central arteries. Stated differently, measuring systemic PWV or carotid artery stiffness may not provide early indicators of altered vascular health.

Reference List

1. **Ahlstrom C, Johansson A, Uhlin F, Lanne T and Ask P**. Noninvasive investigation of blood pressure changes using the pulse wave transit time: a novel approach in the monitoring of hemodialysis patients. *Journal of Artificial Organs* 8: 192-197, 2005.

2. **Aizawa K and Petrella RJ**. Acute and chronic impact of dynamic exercise on arterial stiffness in older hypertensives. *The Open Cardiovascular Medicine Journal* 2: 3, 2008.

3. **Aizawa K, Shoemaker JK, Overend TJ and Petrella RJ**. Effects of lifestyle modification on central artery stiffness in metabolic syndrome subjects with pre-hypertension and/or pre-diabetes. *Diabetes research and clinical practice* 83: 249-256, 2009.

4. **Aldrich JE**. Basic physics of ultrasound imaging. *Critical care medicine* 35: S131, 2007.

5. **Ambring A, Benthin G, Petersson AS, Jungersten L and Wennmalm A**. Indirect evidence of increased expression of NO synthase in marathon runners, and upregulation of NO synthase activity during running. *Circulation* 90: 1-137, 1994.

6. **Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, Target R and Levy BI**. Assessment of arterial distensibility by automatic pulse wave velocity measurement: validation and clinical application studies. *Hypertension* 26: 485, 1995.

7. **Astrup P**. Carbon monoxide and peripheral arterial disease. *Scandinavian journal of clinical and laboratory investigation Supplementum* 99: 193, 1967.

8. **Astrup P, Kjeldsen K and Wanstrup J**. Effects of carbon monoxide exposure on the arterial walls. *Annals of the New York Academy of Sciences* 174: 294-300, 1970.

9. **Auerbach O, Carter HW, Garfinkel L and Hammond EC**. Cigarette smoking and coronary artery disease. A macroscopic and microscopic study. *Chest* 70: 697, 1976.

10. **Auerbach O, Hammond EC and Garfinkel L**. Thickening of walls of arterioles and small arteries in relation to age and smoking habits. *The New England journal of medicine* 278: 980, 1968.

11. **Avolio A, Jones D and Tafazzoli-Shadpour M**. Quantification of alterations in structure and function of elastin in the arterial media. *Hypertension* 32: 170, 1998.

12. **Avolio AP**. Multi-branched model of the human arterial system. *Medical and Biological Engineering and Computing* 18: 709-718, 1980.

13. **Baldassarre D, Castelnuovo S, Frigerio B, Amato M, Werba JP, De Jong A, Ravani AL, Tremoli E and Sirtori CR**. Effects of timing and extent of smoking, type of cigarettes, and concomitant risk factors on the association between smoking and subclinical atherosclerosis. *Stroke* 40: 1991, 2009.

14. **Ballantyne C and Nambi V**. Markers of inflammation and their clinical significance. *Atherosclerosis Supplements* 6: 21-29, 2005.

15. **Bank AJ, Kaiser DR, Rajala S and Cheng A**. In vivo human brachial artery elastic mechanics: effects of smooth muscle relaxation. *Circulation* 100: 41, 1999.

16. **Bank AJ, Wang H, Holte JE, Mullen K, Shammas R and Kubo SH**. Contribution of collagen, elastin, and smooth muscle to in vivo human brachial artery wall stress and elastic modulus. *Circulation* 94: 3263, 1996.

17. **Barenbrock M, Spieker C, Witta J, Evers S, Hoeks APG, Rahn KH and Zidek W**. Reduced distensibility of the common carotid artery in patients treated with ergotamine. *Hypertension* 28: 115, 1996.

18. **Baron JA**. Smoking and estrogen-related disease. *American journal of epidemiology* 119: 9, 1984.

19. **Baron JA, La Vecchia C and Levi F**. The antiestrogenic effect of cigarette smoking in women. *American journal of obstetrics and gynecology* 162: 502, 1990.

20. **Barua RS, Ambrose JA, Eales-Reynolds LJ, DeVoe MC, Zervas JG and Saha DC**. Dysfunctional endothelial nitric oxide biosynthesis in healthy smokers with impaired endothelium-dependent vasodilatation. *Circulation* 104: 1905, 2001.

21. **Barua RS, Ambrose JA, Eales-Reynolds LJ, DeVoe MC, Zervas JG and Saha DC**. Heavy and light cigarette smokers have similar dysfunction of endothelial vasoregulatory activity: an in vivo and in vitro correlation. *Journal of the American College of Cardiology* 39: 1758, 2002.

22. **Bayliss WM**. On the local reactions of the arterial wall to changes of internal pressure. *The Journal of Physiology* 28: 220, 1902.

23. **Bazzano LA, He J, Muntner P, Vupputuri S and Whelton PK**. Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. *Annals of Internal Medicine* 138: 891, 2003.

24. **Benowitz NL**. The role of nicotine in smoking-related cardiovascular disease. *Preventive medicine* 26: 412-417, 1997.

25. **Bergel DH**. The dynamic elastic properties of the arterial wall. *The Journal of Physiology* 156: 458, 1961.

26. **Bermudez EA, Rifai N, Buring JE, Manson JAE and Ridker PM**. Relation between markers of systemic vascular inflammation and smoking in women. *The American Journal of Cardiology* 89: 1117-1119, 2002.

27. **Bevan JA**. Vascular myogenic or stretch-dependent tone. *Journal of Cardiovascular Pharmacology* 7: S129, 1985.

28. **Blacher J, Asmar R, Djane S, London GM and Safar ME**. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* 33: 1111, 1999.

29. **Bode-Boger SM, B÷ger RH, Schr÷der EP and Fr÷lich JC**. Exercise increases systemic nitric oxide production in men. *Journal of CardiovascularRrisk* 1: 173, 1994.

30. **Bogert LWJ and van Lieshout JJ**. Non invasive pulsatile arterial pressure and stroke volume changes from the human finger. *Experimental Physiology* 90: 437-446, 2005.

31. **Bolego C, Poli A and Paoletti R**. Smoking and gender. *Cardiovascular Research* 53: 568, 2002.

32. **Bos WJW, van Goudoever J, van Montfrans GA, van den Meiracker AH and Wesseling KH**. Reconstruction of brachial artery pressure from noninvasive finger pressure measurements. *Circulation* 94: 1870, 1996.

33. **Boutouyrie P, Lacolley P, Girerd X, Beck L, Safar M and Laurent S**. Sympathetic activation decreases medium-sized arterial compliance in humans. *American Journal of Physiology-Heart and Circulatory Physiology* 267: H1368, 1994.

34. **Brigham EO**. *The fast Fourier transform and its applications*. 1988.

35. **Bruel A, Ïrtoft G and Oxlund H**. Inhibition of cross-links in collagen is associated with reduced stiffness of the aorta in young rats. *Atherosclerosis* 140: 135-145, 1998.

36. **Burattini R and Di Salvia PO**. Development of systemic arterial mechanical properties from infancy to adulthood interpreted by four-element windkessel models. *Journal of Applied Physiology* 103: 66, 2007.

37. **Burton AC**. Relation of structure to function of the tissues of the wall of blood vessels. *Physiological reviews* 34: 619, 1954.

38. **Burton, A. C.** Physiology and biophysics of the circulation. Chicago: Year Book Medical Publishers. 1965. Chicago, Year Book Medical Publishers.

39. **Cameron JD and Dart AM**. Exercise training increases total systemic arterial compliance in humans. *American Journal of Physiology-Heart and Circulatory Physiology* 266: H693, 1994.

40. **Carty CS, Huribal M, Marsan BU, Ricotta JJ and Dryjski M**. Nicotine and its metabolite cotinine are mitogenic for human vascular smooth muscle cells. *Journal of Vascular Surgery* 25: 682-688, 1997.

41. **Cattell MA, Anderson JC and Hasleton PS**. Age-related changes in amounts and concentrations of collagen and elastin in normotensive human thoracic aorta. *Clinica Chimica Acta* 245: 73-84, 1996.

42. **Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J and Deanfield JE**. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* 88: 2149, 1993.

43. **Chelsky R, Wilson RA, Morton MJ, Burry KA, Patton PE, Szumowski J and Giraud GD**. Alteration of ascending thoracic aorta compliance after treatment with menotropin. *American Journal of Obstetrics and Gynecology* 176: 1255-1261, 1997.

44. **Cheng KS, Mikhailidis DP, Hamilton G and Seifalian AM**. A review of the carotid and femoral intima-media thickness as an indicator of the presence of peripheral vascular disease and cardiovascular risk factors. *Cardiovascular Research* 54: 528-538, 2002.

45. **Cortez-Cooper MY, DeVan AE, Anton MM, Farrar RP, Beckwith KA, Todd JS and Tanaka H**. Effects of high intensity resistance training on arterial stiffness and wave reflection in women. *American Journal of Hypertension* 18: 930-934, 2005.

46. **Cox RH, Tulenko T and Santamore WP**. Effects of chronic cigarette smoking on canine arteries. *Am J Physiol Heart Circ Physiol* 246: H97-103, 1984.

47. **Diezroux AV, Nieto FJ, Comstock GW, Howard G and Szklo M**. The relationship of active and passive smoking to carotid atherosclerosis 12-14 years later. *Preventive Medicine* 24: 48-55, 1995.

48. **Dinenno FA, Tanaka H, Monahan KD, Clevenger CM, Eskurza I, DeSouza CA and Seals DR**. Regular endurance exercise induces expansive arterial remodelling in the trained limbs of healthy men. *The Journal of Physiology* 534: 287-295, 2001.

49. **Dobrin PB**. Mechanics of normal and diseased blood vessels. *Annals of Vascular Surgery* 2: 283-294, 1988.

50. **Dobrin PB and Rovick AA**. Influence of vascular smooth muscle on contractile mechanics and elasticity of arteries. *American Journal of Physiology--Legacy Content* 217: 1644, 1969.

51. **Doll R, Peto R, Wheatley K, Gray R and Sutherland I**. Mortality in relation to smoking: 40 years' observations on male British doctors. *BMJ* 309: 901, 1994.

52. **Duckles SP, Krause DN, Stirone C and Procaccio V**. Estrogen and Mitochondria: A New Paradigm for Vascular Protection? *Molecular Interventions* 6: 26, 2006.

53. **Faber JE**. In situ analysis of alpha-adrenoceptors on arteriolar and venular smooth muscle in rat skeletal muscle microcirculation. *Circulation Research* 62: 37, 1988.

54. **Failla M, Grappiolo A, Carugo S, Calchera I, Giannattasio C and Mancia G**. Effects of cigarette smoking on carotid and radial artery distensibility. *Journal of Hypertension* 15: 1659, 1997.

55. **Fisher GM and Laurado JG**. Collagen and elastin content in canine arteries selected from functionally different vascular beds. *Circulation Research* 19: 394, 1966.

56. **Fishman AP**. Endothelium: a distributed organ of diverse capabilities. *Annals of the New York Academy of Sciences* 401: 1-8, 1982.

57. **Fonck E, Prod'hom G, Roy S, Augsburger L, Rufenacht DA and Stergiopulos N**. Effect of elastin degradation on carotid wall mechanics as assessed by a constituent-based biomechanical model. *American Journal of Physiology - Heart and Circulatory Physiology* 292: H2754-H2763, 2007.

58. **Frances MF**. *Impact of alpha adrenergic and myogenic control on forearm vasomotor properties* (Master of Science, Dissertation). The University of Western Ontario, 2009.

59. **Franklin SS, Khan SA, Wong ND, Larson MG and Levy D**. Is pulse pressure useful in predicting risk for coronary heart disease?: The Framingham Heart Study. *Circulation* 100: 354, 1999.

60. **Furchgott RF**. Role of endothelium in responses of vascular smooth muscle. *Circulation Research* 53: 557, 1983.

61. **Gates PE and Seals DR**. Decline in large elastic artery compliance with age: a therapeutic target for habitual exercise. *British Journal of Sports Medicine* 40: 897, 2006.

62. **Giannattasio C, Failla M, Grappiolo A, Calchera I, Grieco N, Carugo S, Bigoni M, Randelli P, Peretti G and Mancia G**. Effects of physical training of the dominant arm on ipsilateral radial artery distensibility and structure. *Journal of Hypertension* 19: 71, 2001.

63. **Gill RW**. Measurement of blood flow by ultrasound: accuracy and sources of error. *Ultrasound in Medicine & Biology* 11: 625-641, 1985.

64. **Godia EC, Madhok R, Pittman J, Trocio S, Ramas R, Cabral D, Sacco RL and Rundek T**. Carotid Artery Distensibility: A Reliability Study. *J Ultrasound Med* 26: 1157-1165, 2007.

65. **Green CR and Rodgman A**. The Tobacco Chemists' Research Conference. A half-century of advances in analytical methodology of tobacco and its products. *Recent Advances in Tobacco Science* 22: 131-304, 1996.

66. **Green DJ, Walsh JH, Maiorana A, Burke V, Taylor RR and O'Driscoll JG**. Comparison of resistance and conduit vessel nitric oxide-mediated vascular function in vivo: effects of exercise training. *Journal of Applied Physiology* 97: 749, 2004.

67. **Greenwald SE, Newman DL and Denyer HT**. Effect of smooth muscle activity on the static and dynamic elastic properties of the rabbit carotid artery. *Cardiovascular Research* 16: 86, 1982.

68. **Guelen I, Westerhof BE, van der Sar GL, van Montfrans GA, Kiemeneij F, Wesseling KH and Bos WJW**. Finometer, finger pressure measurements with the possibility to reconstruct brachial pressure. *Blood Pressure Monitoring* 8: 27, 2003.

69. **Guelen I, Westerhof BE, van der Sar GL, van Montfrans GA, Kiemeneij F, Wesseling KH and Bos WJW**. Validation of brachial artery pressure reconstruction from finger arterial pressure. *Journal of Hypertension* 26: 1321, 2008.

70. **Guyton AC and Hall JE**. Textbook of medical physiology. *London: W B Saunders Co* 1032, 1981.

71. **Hackam DG and Anand SS**. Emerging Risk Factors for Atherosclerotic Vascular Disease. *JAMA* 290: 932-940, 2003.

72. **Halliwill JR and Minson CT**. Effect of hypoxia on arterial baroreflex control of heart rate and muscle sympathetic nerve activity in humans. *Journal of Applied Physiology* 93: 857, 2002.

73. **Halvorsen B, Sagen LE, Ueland T, Aukrust P and Tonstad S**. Effect of smoking cessation on markers of inflammation and endothelial cell activation among individuals with high risk for cardiovascular disease. *Scandinavian Journal of Clinical & Laboratory Investigation* 67: 604-611, 2007.

74. **Hamilton P, Lockhart C, Quinn C and Mcveigh G**. Arterial stiffness: clinical relevance, measurement and treatment. *Clinical Science* 113: 157-170, 2007.

75. **Harlow BL and Signorello LB**. Factors associated with early menopause. *Maturitas* 35: 3-9, 2000.

76. **Hashimoto M, Akishita M, Eto M, Ishikawa M, Kozaki K, Toba K, Sagara Y, Taketani Y, Orimo H and Ouchi Y**. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. *Circulation* 92: 3431, 1995.

77. **Hayashi T, Matsui-Hirai H, Miyazaki-Akita A, Fukatsu A, Funami J, Ding QF, Kamalanathan S, Hattori Y, Ignarro LJ and Iguchi A**. Endothelial cellular senescence is inhibited by nitric oxide: implications in atherosclerosis associated with menopause and diabetes. *Proceedings of the National Academy of Sciences* 103: 17018, 2006.

78. **Howard G, Wagenknecht LE, Burke GL, Diez-Roux A, Evans GW, McGovern P, Nieto FJ and Tell GS**. Cigarette smoking and progression of atherosclerosis: The Atherosclerosis Risk in Communities (ARIC) Study. *JAMA* 279: 119, 1998.

79. **Ignarro LJ, Buga GM, Wood KS, Byrns RE and Chaudhuri G**. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proceedings of the National Academy of Sciences of the United States of America* 84: 9265, 1987.

80. **Ijzerman RG, Serne EH, van Weissenbruch MM, de Jongh RT and Stehouwer CDA**. Cigarette smoking is associated with an acute impairment of microvascular function in humans. *Clinical Science* 104: 247-252, 2003.

81. **Imholz BPM, Wieling W, van Montfrans GA and Wesseling KH**. Fifteen years experience with finger arterial pressure monitoring. *Cardiovascular Research* 38: 605-616, 1998.

82. **Imrich R, Eldadah BA, Bentho O, Pechnik S, Sharabi Y, Holmes C and Goldstein DS**. Attenuated Pre ejection Period Response to Tyramine in Patients with Cardiac Sympathetic Denervation. *Annals of the New York Academy of Sciences* 1148: 486-489, 2008.

83. **Izzo Jr JL, Manning TS and Shykoff BE**. Office blood pressures, arterial compliance characteristics, and estimated cardiac load. *Hypertension* 38: 1467, 2001.

84. **Jacobs Jr DR, Adachi H, Mulder I, Kromhout D, Menotti A, Nissinen A and Blackburn H**. Cigarette smoking and mortality risk: twenty-five-year follow-up of the Seven Countries Study. *Archives of Internal Medicine* 159: 733, 1999.

85. **Jaggar JH, Wellman GC, Heppner TJ, Porter VA, Perez GJ, Gollasch M, Kleppisch T, Rubart M, Stevenson AS and Lederer WJ**. Ca2+ channels, ryanodine receptors and Ca2+-activated K+ channels: a functional unit for regulating arterial tone. *Acta physiologica scandinavica* 164: 577-587, 1998.

86. **Jansen JRC, Schreuder JJ, Mulier JP, Smith NT, Settels JJ and Wesseling KH**. A comparison of cardiac output derived from the arterial pressure wave against thermodilution in cardiac surgery patients. *British Journal of Anaesthesia* 87: 212, 2001.

87. **Jatoi NA, Jerrard-Dunne P, Feely J and Mahmud A**. Impact of smoking and smoking cessation on arterial stiffness and aortic wave reflection in hypertension. *Hypertension* 49: 981, 2007.

88. **Jung ME, Fitzgeorge L, Prapavessis H, Faulkner G and Maddison R**. The getting physical on cigarettes trial: rationale and methods. *Mental Health and Physical Activity* 3: 35-44, 2010.

89. **Kablak-Ziembicka A, Tracz W, Przewlocki T, Pieniazek P, Sokolowski A and Konieczynska M**. Association of increased carotid intima-media thickness with the extent of coronary artery disease. *Heart* 90: 1286, 2004.

90. **Kawachi I, Colditz GA, Stampfer MJ, Willett WC, Manson JAE, Rosner B, Hunter DJ, Hennekens CH and Speizer FE**. Smoking cessation in relation to total mortality rates in women: a prospective cohort study. *Annals of Internal Medicine* 119: 992, 1993.

91. **Kawaskit T, Sasayama S, Yagisi SI, Asakawa T and Hirai T**. Non-invasive assessment of the age related changes in stiffness of major branches of the human arteries. *Cardiovascular Research* 21: 678, 1987.

92. **Kawecka-Jaszcz K, Czarnecka D, Olszanecka A, Rajzer M and Jankowski P**. The effect of hormone replacement therapy on arterial blood pressure and vascular compliance in postmenopausal women with arterial hypertension. *Journal of human hypertension* 16: 509, 2002.

93. **Kember GC, Zamir M and Armour JA**. Smart baroreception along the aortic arch, with reference to essential hypertension. *Physical Review E* 70: 51914, 2004.

94. **Keys A**. *Seven countries. A multivariate analysis of death and coronary heart disease*. Harvard University Press, 1980.

95. **Kim JW, Park CG, Hong SJ, Park SM, Rha SW, Seo HS, Oh DJ and Rho YM**. Acute and chronic effects of cigarette smoking on arterial stiffness. *Blood Pressure* 14: 80-85, 2005.

96. **Kingwell BA and Gatzka CD**. Arterial stiffness and prediction of cardiovascular risk. *Journal of Hypertension* 20: 2337, 2002.

97. **Kool MJF, Hoeks APG, Struijker Boudier HAJ, Reneman RS and Van Bortel LMAB**. Short and long-term effects of smoking on arterial wall properties in habitual smokers. *Journal of the American College of Cardiology* 22: 1881-1886, 1993.

98. **Kubozono T, Miyata M, Ueyama K, Hamasaki S, Kusano K, Kubozono O and Tei C**. Acute and Chronic Effects of Smoking on Arterial Stiffness. *Circulation Journal* 75: 698-702, 2011.

99. **Kugiyama K, Yasue H, Ohgushi M, Motoyama T, Kawano H, Inobe Y, Hirashima O and Sugiyama S**. Deficiency in nitric oxide bioactivity in epicardial coronary arteries of cigarette smokers. *Journal of the American College of Cardiology* 28: 1161-1167, 1996.

100. **Lakatta EG and Levy D**. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a" set up" for vascular disease. *Circulation* 107: 139, 2003.

101. **Langewouters GJ, Wesseling KH and Goedhard WJA**. The static elastic properties of 45 human thoracic and 20 abdominal aortas in vitro and the parameters of a new model. *Journal of Biomechanics* 17: 425-435, 1984.

102. **Laurent S, Caviezel B, Beck L, Girerd X, Billaud E, Boutouyrie P, Hoeks A and Safar M**. Carotid artery distensibility and distending pressure in hypertensive humans. *Hypertension* 23: 878, 1994.

103. **Leaf DA and Kleinman MT**. Acute exposure to carbon monoxide does not affect plasma lipids, lipoproteins, and apolipoproteins. *Angiology* 47: 337, 1996.

104. **Lekakis J, Papamichael C, Vemmos C, Nanas J, Kontoyannis D, Stamatelopoulos S and Moulopoulos S**. Effect of acute cigarette smoking on endothelium-dependent brachial artery dilatation in healthy individuals. *The American Journal of Cardiology* 79: 529, 1997.

105. **Leuenberger U, Gleeson K, Wroblewski K, Prophet S, Zelis R, Zwillich C and Sinoway L**. Norepinephrine clearance is increased during acute hypoxemia in humans. *American Journal of Physiology-Heart and Circulatory Physiology* 261: H1659, 1991.

106. **Lew R, Komesaroff P, Williams M, Dawood T and Sudhir K**. Endogenous estrogens influence endothelial function in young men. *Circulation Research* 93: 1127, 2003.

107. **Liang YL, Shiel LM, Teede H, Kotsopoulos D, McNeil J, Cameron JD and McGrath BP**. Effects of blood pressure, smoking, and their interaction on carotid artery structure and function. *Hypertension* 37: 6, 2001.

108. **Libby P, Ridker PM and Maseri A**. Inflammation and atherosclerosis. *Circulation* 105: 1135, 2002.

109. **London GM and Guerin AP**. Influence of arterial pulse and reflected waves on blood pressure and cardiac function. *American Heart Journal* 138: S220-S224, 1999.

110. **Lott MEJ, Herr MD and Sinoway LI**. Effects of age on brachial artery myogenic responses in humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 287: R586, 2004.

111. **Lydrup ML and Ferno M**. Correlation between estrogen receptor alpha expression, collagen content and stiffness in human uterine arteries. *Acta Obstetricia et Gynecologica Scandinavica* 82: 610-615, 2003.

112. **Mahmud A and Feely J**. Effect of smoking on arterial stiffness and pulse pressure amplification. *Hypertension* 41: 183, 2003.

113. **Marcus BH, Albrecht AE, King TK, Parisi AF, Pinto BM, Roberts M, Niaura RS and Abrams DB**. The efficacy of exercise as an aid for smoking cessation in women: a randomized controlled trial. *Archives of Internal Medicine* 159: 1229, 1999.

114. **Masi CM, Hawkley LC, Berry JD and Cacioppo JT**. Estrogen metabolites and systolic blood pressure in a population-based sample of postmenopausal women. *Journal of Clinical Endocrinology & Metabolism* 91: 1015, 2006.

115. **Mattace-Raso FUS, van der Cammen TJM, Hofman A, van Popele NM, Bos ML, Schalekamp MADH, Asmar R, Reneman RS, Hoeks APG, Breteler MMB and Witteman JCM**. Arterial Stiffness and Risk of Coronary Heart Disease and Stroke: The Rotterdam Study. *Circulation* 113: 657-663, 2006.

116. **Mattar L**. *Early Onset Vascular Stiffening and Sexual Dimorphism of Sympathetic Control in the Spontaneously Hypertensive Rat* (PhD, Dissertation). The University of Western Ontario, 2011.

117. **Mayhan WG and Patel KP**. Effect of nicotine on endothelium-dependent arteriolar dilatation in vivo. *American Journal of Physiology- Heart and Circulatory Physiology* 272: H2337, 1997.

118. **McDonough P and Moffatt RJ**. Smoking-induced elevations in blood carboxyhaemoglobin levels: effect on maximal oxygen uptake. *Sports Medicine* 27: 275-283, 1999.

119. **McGill HC**. The cardiovascular pathology of smoking* 1. *American Heart Journal* 115: 250-257, 1988.

120. **McVeigh GE, Finklestein SM and Cohn JN**. Pulse contour and impedance parameters derived from arterial waveform analysis. *Functional Abnormalities of the Aorta (Boudoulas, H , Toutouzas, P and Wooley, CF, eds)* 183-193, 1996.

121. **McVeigh GE, Lemay L, Morgan D and Cohn JN**. Effects of long-term cigarette smoking on endothelium-dependent responses in humans. *The American Journal of Cardiology* 78: 668, 1996.

122. **McVeigh GE, Morgan DJ, Finkelstein SM, Lemay LA and Cohn JN**. Vascular abnormalities associated with long-term cigarette smoking identified by arterial waveform analysis. *The American Journal of Medicine* 102: 227-231, 1997.

123. **Meek MD and Finch GL**. Diluted Mainstream Cigarette Smoke Condensates Activate Estrogen Receptor and Aryl Hydrocarbon Receptor-Mediated Gene Transcription* 1. *Environmental Research* 80: 9-17, 1999.

124. **Meire HB and Farrant P**. *Basic Ultrasound*. Great Britain: John Wiley & Sons, Ltd., 1995.

125. **Meredith IT, Friberg P, Jennings GL, Dewar EM, Fazio VA, Lambert GW and Esler MD**. Exercise training lowers resting renal but not cardiac sympathetic activity in humans. *Hypertension* 18: 575, 1991.

126. **Messerli FH, Frohlich ED and Ventura HO**. Arterial compliance in essential hypertension. *Journal of Cardiovascular Pharmacology* 7: S33, 1985.

127. **Middleton ET and Morice AH**. Breath Carbon Monoxide as an Indication of Smoking Habit*. *Chest* 117: 758, 2000.

128. **Miller VM and Duckles SP**. Vascular actions of estrogens: functional implications. *Pharmacological Reviews* 60: 210, 2008.

129. **Minami J, Ishimitsu T and Matsuoka H**. Effects of Smoking Cessation on Blood Pressure and Heart Rate Variability in Habitual Smokers. *Hypertension* 33: 586-590, 1999.

130. **Mitchell DG**. Color Doppler imaging: principles, limitations, and artifacts. *Radiology* 177: 1, 1990.

131. **Morgan BJ**. Vascular consequences of intermittent hypoxia. *Hypoxia and the Circulation* 69-84, 2007.

132. **Mueller PJ**. Exercise training attenuates increases in lumbar sympathetic nerve activity produced by stimulation of the rostral ventrolateral medulla. *Journal of Applied Physiology* 102: 803, 2007.

133. **Myers AH, Rosner B, Abbey H, Willet W, Stampfer MJ, Bain C, Lipnick R, Hennekens C and Speizer F**. Smoking behavior among participants in the nurses' health study. *American Journal of Public Health* 77: 628, 1987.

134. **Napoli C and Ignarro LJ**. Nitric oxide and atherosclerosis. *Nitric Oxide* 5: 88-97, 2001.

135. **Narkiewicz K, van de Borne PJH, Hausberg M, Cooley RL, Winniford MD, Davison DE and Somers VK**. Cigarette Smoking Increases Sympathetic Outflow in Humans. *Circulation* 98: 528-534, 1998.

136. **Nedeljkovic ZS, Gokce N and Loscalzo J**. Mechanisms of oxidative stress and vascular dysfunction. *Postgraduate Medical Journal* 79: 195, 2003.

137. **Nichols WW and Michael FOR**. *McDonald's blood flow in arteries: theoretical, experimental and clinical principles*. Hodder Arnold, 2005.

138. **Nilsson H, Goldstein M and Nilsson OLA**. Adrenergic innervation and neurogenic response in large and small arteries and veins from the rat. *Acta physiologica scandinavica* 126: 121-133, 1986.

139. **Njolstad I, Arnesen E and Lund-Larsen PG**. Smoking, serum lipids, blood pressure, and sex differences in myocardial infarction: a 12-year follow-up of the Finnmark Study. *Circulation* 93: 450, 1996.

140. **Noble A, Johnson R, Thomas A and Bass P**. *The Cardiovascular System: Basic Science and Clinical Conditions. Second Edition*. China: Elsevier, 2010.

141. **Node K, Kitakaze M, Yoshikawa H, Kosaka H and Hori M**. Reversible reduction in plasma concentration of nitric oxide induced by cigarette smoking in young adults. *The American Journal of Cardiology* 79: 1538, 1997.

142. **Noon JP**. The Arterial Pulse Wave and Vascular Compliance. *Progress in Cardiovascular Nursing* 24: 53-58, 2009.

143. **O'Rourke M**. Arterial stiffness, systolic blood pressure, and logical treatment of arterial hypertension. *Hypertension* 15: 339, 1990.

144. **O'Rourke MF**. Isolated systolic hypertension, pulse pressure, and arterial stiffness as risk factors for cardiovascular disease. *Current Hypertension Reports* 1: 204-211, 1999.

145. **O'Rourke MF, Staessen JA, Vlachopoulos C, Duprez D and Plante Gθ**. Clinical applications of arterial stiffness; definitions and reference values. *American Journal of Hypertension* 15: 426-444, 2002.

146. **Oncken CA, White WB, Cooney JL, Van Kirk JR, Ahluwalia JS and Giacco S**. Impact of smoking cessation on ambulatory blood pressure and heart rate in postmenopausal women. *American Journal of Hypertension* 14: 942-949, 2001.

147. **Ota Y, Kugiyama K, Sugiyama S, Ohgushi M, Matsumura T, Doi H, Ogata N, Oka H and Yasue H**. Impairment of endothelium-dependent relaxation of rabbit aortas by cigarette smoke extract--role of free radicals and attenuation by captopril. *Atherosclerosis* 131: 195-202, 1997.

148. **Planas A, Clarβ A, Marrugat J, Pon JM, Gasol A, de Moner A, Contreras C and Vidal-Barraquer F**. Age at onset of smoking is an independent risk factor in peripheral artery disease development*. *Journal of Vascular Surgery* 35: 506-509, 2002.

149. **PoredoÜ P, Orehek M and Tratnik E**. Smoking is associated with dose-related increase of intima-media thickness and endothelial dysfunction. *Angiology* 50: 201, 1999.

150. **Powell JT**. Vascular damage from smoking: disease mechanisms at the arterial wall. *Vascular Medicine* 3: 21, 1998.

151. **Prapavessis H, Cameron L, Baldi JC, Robinson S, Borrie K, Harper T and Grove JR**. The effects of exercise and nicotine replacement therapy on smoking rates in women. *Addictive Behaviors* 32: 1416-1432, 2007.

152. **Pryor WA and Stone K**. Oxidants in Cigarette Smoke Radicals, Hydrogen Peroxide, Peroxynitrate, and Peroxynitritea. *Annals of the New York Academy of Sciences* 686: 12-27, 1993.

153. **Public Health Agency of Canada**. Cardiovascular Disease Morbidity, Mortality, and Risk Factors Surveillance Information. 2009.

154. **Raij L, DeMaster EG and Jaimes EA**. Cigarette smoke-induced endothelium dysfunction: role of superoxide anion. *Journal of Hypertension* 19: 891, 2001.

155. **Rajkumar MD**. Hormonal therapy increases arterial compliance in postmenopausal women. *Journal of the American College of Cardiology* 30: 350-356, 1997.

156. **Rehill N, Beck CR, Yeo KR and Yeo WW**. The effect of chronic tobacco smoking on arterial stiffness. *British journal of clinical pharmacology* 61: 767-773, 2006.

157. **Rehm J, Baliunas D, Brochu S, Fischer B, Gnam W, Patra J, Popova S, Sarnocinska-Hart A, Taylor B and Adlaf E**. The costs of substance abuse in Canada 2002. *Ottawa: Canadian Centre on Substance Abuse* 2006.

158. **Reneman RS, van Merode T, Brands PJ and Hoeks APG**. Inhomogeneities in arterial wall properties under normal and pathological conditions. *Journal of Hypertension* 10: S35, 1992.

159. **Ross R**. The pathogenesis of atherosclerosisùan update. *New England Journal of Medicine* 314: 488-500, 1986.

160. **Roveda F, Middlekauff HR, Rondon MU, Reis SF, Souza M, Nastari L, Barretto AC, Krieger EM and Negrπo CE**. The effects of exercise training on sympathetic neural activation in advanced heart failure: A randomized controlled trial. *Journal of the American College of Cardiology* 42: 854-860, 2003.

161. **Rowell LB**. *Human cardiovascular control*. Oxford University Press, USA, 1993.

162. **Safar ME, Levy BI and Struijker-Boudier H**. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. *Circulation* 107: 2864, 2003.

163. **Sagawa K, Lie RK and Schaefer J**. Translation of Otto Frank's paper" Die Grundform des Arteriellen Pulses" Zeitschrift fur Biologie 37: 483-526 (1899). *Journal of Molecular and Cellular Cardiology* 22: 253, 1990.

164. **Salonen JT and Salonen R**. Quantitative imaging, risk factors, prevalence, and change: chairmanÆs discussion of session 2: ultrasound b-mode imaging in observational studies of atherosclerotic progression. *Circulation* 87: 1993.

165. **Salzer DA, Medeiros PJ, Craen R and Shoemaker JK**. Neurogenic-nitric oxide interactions affecting brachial artery mechanics in humans: roles of vessel distensibility vs. diameter. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 295: R1181, 2008.

166. **Sanz J and Fayad ZA**. Imaging of atherosclerotic cardiovascular disease. *Nature* 451: 953-957, 2008.

167. **Schubert R, Lidington D and Bolz SS**. The emerging role of Ca2+ sensitivity regulation in promoting myogenic vasoconstrictionå. *Cardiovascular Research* 77: 8, 2008.

168. **Schwartz CJ, Wethessen NT and Wolf S**. *Structure and Function of the Circulation. Volume 3*. New York: Plenum Press, 1981.

169. **Schwartz SM, Campbell GR and Campbell JH**. Replication of smooth muscle cells in vascular disease. *Circulation Research* 58: 427-444, 1986.

170. **Shipley RE, Gregg DE and Schroeder EF**. An experimental stufy of flow patterns in various peripheral arteries. *American Journal of Physiology* 138: 718-730, 1943.

171. **Shoemaker JK, Pozeg ZI and Hughson RL**. Forearm blood flow by Doppler ultrasound during rest and exercise: tests of day-to-day repeatability. *Medicine & Science in Sports & Exercise* 28: 1144, 1996.

172. **Siegman MJ, Butler TM, Mooers SU and Davies RE**. Calcium-dependent resistance to stretch and stress relaxation in resting smooth muscles. *American Journal of Physiology--Legacy Content* 231: 1501, 1976.

173. **Siegman MJ, Butler TM, Mooers SU and Davies RE**. Crossbridge attachment, resistance to stretch, and viscoelasticity in resting mammalian smooth muscle. *Science* 191: 383, 1976.

174. **Silke B and McAuley D**. Accuracy and precision of blood pressure determination with the Finapres: an overview using re-sampling statistics. *Journal of Human Hypertension* 12: 403-409, 1998.

175. **Smith CJ and Fischer TH**. Particulate and vapor phase constituents of cigarette mainstream smoke and risk of myocardial infarction. *Atherosclerosis* 158: 257-267, 2001.

176. **Somers VK, Mark AL, Zavala DC and Abboud FM**. Contrasting effects of hypoxia and hypercapnia on ventilation and sympathetic activity in humans. *Journal of Applied Physiology* 67: 2101, 1989.

177. **Spina M and Garbin G**. Age-related chemical changes in human elastins from non-atherosclerotic areas of thoracic aorta* 1. *Atherosclerosis* 24: 267-279, 1976.

178. **Stamatelopoulos KS, Kalpakos D, Protogerou AD, Papamichael CM, Ikonomidis I, Tsitsirikos M, Revela I, Papaioannou TG and Lekakis JP**. The combined effect of augmentation index and carotid intima-media thickness on cardiovascular risk in young and middle-aged men without cardiovascular disease. *Journal of Human Hypertension* 20: 273-279, 2006.

179. **Stehbens WE**. *Hemodynamics and the Blood Vessel Wall*. Charles C. Thomas Publisher, 1979.

180. **Steinback CD, Salzer D, Medeiros PJ, Kowalchuk J and Shoemaker JK**. Hypercapnic vs. hypoxic control of cardiovascular, cardiovagal, and sympathetic function. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 296: R402, 2009.

181. **Stergiopulos N, Meister JJ and Westerhof N**. Evaluation of methods for estimation of total arterial compliance. *American Journal of Physiology-Heart and Circulatory Physiology* 268: H1540, 1995.

182. **Stone PJ, Gottlieb DJ, O'Connor GT, Ciccolella DE, Breuer R, Bryan-Rhadfi J, Shaw HA, Franzblau C and Snider GL**. Elastin and collagen degradation products in urine of smokers with and without chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 151: 952-959, 1995.

183. **Strand, F. L**. Physiology: a regulatory systems approach, ed. 2, New York, 1983. Macmillan Publishing Co.

184. **Swierblewska E, Hering D, Kara T, Kunicka K, Kruszewski P, Bieniaszewski L, Boutouyrie P, Somers VK and Narkiewicz K**. An independent relationship between muscle sympathetic nerve activity and pulse wave velocity in normal humans. *Journal of Hypertension* 28: 979, 2010.

185. **Tanaka H, Dinenno FA, Monahan KD, Clevenger CM, DeSouza CA and Seals DR**. Aging, habitual exercise, and dynamic arterial compliance. *Circulation* 102: 1270, 2000.

186. **Tatchum-Talom R, Martel C and Marette A**. Influence of estrogen on aortic stiffness and endothelial function in female rats. *American Journal of Physiology-Heart and Circulatory Physiology* 282: H491, 2002.

187. **Taylor KJ and Holland S**. Doppler US. Part I. Basic principles, instrumentation, and pitfalls. *Radiology* 174: 297, 1990.

188. **Teo KK, Ounpuu S, Hawken S, Pandey MR, Valentin V, Hunt D, Diaz R, Rashed W, Freeman R and Jiang L**. Tobacco use and risk of myocardial infarction in 52 countries in the INTERHEART study: a case-control study. *The Lancet* 368: 647-658, 2006.

189. **Thrush A and Hartshorne T**. *Peripheral Vascular Ultrasound*. Churchill: Elsevier, 2005.

190. **Tomiyama H, Hashimoto H, Tanaka H, Matsumoto C, Odaira M, Yamada J, Yoshida M, Shiina K, Nagata M and Yamashina A**. Continuous Smoking and Progression of Arterial Stiffening: A Prospective Study. *Journal of the American College of Cardiology* 55: 1979, 2010.

191. **Tomiyama H, Yamashina A, Arai T, Hirose K, Koji Y, Chikamori T, Hori S, Yamamoto Y, Doba N and Hinohara S**. Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement--a survey of 12 517 subjects. *Atherosclerosis* 166: 303-309, 2003.

192. **Tsiara S, Elisaf M and Mikhailidis DP**. Influence of smoking on predictors of vascular disease. *Angiology* 54: 507, 2003.

193. **Vaitkevicius PV, Fleg JL, Engel JH, O'Connor FC, Wright JG, Lakatta LE, Yin FC and Lakatta EG**. Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation* 88: 1456, 1993.

194. **Van Bortel L**. Focus on small artery stiffness. *Journal of Hypertension* 20: 1707, 2002.

195. **Van den Berkmortel F, Wollersheim H, van Langen H, de Boo T and Thien T**. Dynamic vessel wall properties of large conduit arteries in habitual cigarette smokers. *European Journal of Internal Medicine* 10: 159-165, 1999.

196. **Vanhoutte PM**. Endothelial dysfunction and atherosclerosis. *European heart Journal* 18: 19, 1997.

197. **Villablanca AC, Jayachandran M and Banka C**. Atherosclerosis and sex hormones: current concepts. *Clinical Science* 119: 493-513, 2010.

198. **Watts DT**. The effect of nicotine and smoking on the secretion of epinephrine. *Annals of the New York Academy of Sciences* 90: 74-80, 1960.

199. **Weil BR, Byrne SA, Baynard T and Kanaley JA**. Vascular function is impaired early after the initiation of chronic cigarette smoking. *Artery Research* 3: 111-114, 2009.

200. **Wesseling KH, Jansen JR, Settels JJ and Schreuder JJ**. Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *Journal of Applied Physiology* 74: 2566, 1993.

201. **Westerhof N, Lankhaar JW and Westerhof BE**. The arterial windkessel. *Medical and Biological Engineering and Computing* 47: 131-141, 2009.

202. **Wetterer E**. Flow and pressure in the arterial system, their hemodynamic relationship, and the principles of their measurement. *Minnesota Medicine* 37: 77, 1954.

203. **Whisnant JP, Homer D, Ingall TJ, Baker Jr HL, O'Fallon WM and Wievers DO**. Duration of cigarette smoking is the strongest predictor of severe extracranial carotid artery atherosclerosis. *Stroke* 21: 707, 1990.

204. **Wiesmann F, Petersen SE, Leeson PM, Francis JM, Robson MD, Wang Q, Choudhury R, Channon KM and Neubauer S**. Global impairment of brachial, carotid, and aortic vascular function in young smokers:: Direct quantification by high-resolution magnetic resonance imaging. *Journal of the American College of Cardiology* 44: 2056-2064, 2004.

205. **Wilson PWF, D'Agostino RB, Levy D, Belanger AM, Silbershatz H and Kannel WB**. Prediction of coronary heart disease using risk factor categories. *Circulation* 97: 1837, 1998.

206. **Witteman JC, Grobbee DE, Valkenburg HA, Van Hemert AM, Stijnen T and Hofman A**. Cigarette smoking and the development and progression of aortic atherosclerosis. A 9-year population-based follow-up study in women. *Circulation* 88: 2156, 1993.

207. **Xu C, Zarins CK, Pannaraj PS, Bassiouny HS and Glagov S**. Hypercholesterolemia superimposed by experimental hypertension induces differential distribution of collagen and elastin. *Arteriosclerosis, Thrombosis, and Vascular Biology* 20: 2566, 2000.

208. **Yoshizawa M, Maeda S, Miyaki A, Misono M, Saito Y, Tanabe K, Kuno S and Ajisaka R**. Effect of 12 weeks of moderate intensity resistance training on arterial stiffness: a randomised controlled trial in women aged 32 – 59 years. *British Journal of Sports Medicine* 43: 615-618, 2009.

209. **Yotsukura M, Koide Y, Fujii K, Tomono Y, Katayama A, Ando H, Suzuki J and Ishikawa K**. Heart rate variability during the first month of smoking cessation. *American Heart Journal* 135: 1004-1009, 1998.

210. **Zamir M**. *The physics of coronary blood flow*. Springer Verlag, 2005.

211. **Zamir M, Goswami R, Salzer D and Shoemaker JK**. Role of vascular bed compliance in vasomotor control in human skeletal muscle. *Experimental physiology* 92: 841-848, 2007.

212. **Zamir M, Norton K, Fleischhauer A, Frances MF, Goswami R, Usselman CW, Nolan RP and Shoemaker JK**. Dynamic responsiveness of the vascular bed as a regulatory mechanism in vasomotor control. *The Journal of general physiology* 134: 69, 2009.

213. **Zeiher AM, Schachinger V and Minners J**. Long-term cigarette smoking impairs endothelium-dependent coronary arterial vasodilator function. *Circulation* 92: 1094, 1995.

214. **Zhang Y, Stewart KG and Davidge ST**. Estrogen replacement reduces age-associated remodeling in rat mesenteric arteries. *Hypertension* 36: 970, 2000.

215. **Zhang Y, Stewart KG and Davidge ST**. Endogenous estrogen mediates vascular reactivity and distensibility in pregnant rat mesenteric arteries. *American Journal of Physiology-Heart and Circulatory Physiology* 280: H956, 2001.

APPENDIX 1 : USE OF HUMAN ETHICAL APPROVAL NOTICE

Office of Research Ethics
The University of Western Ontario
Room 4180 Support Services Building, London, ON. Canada N6A 5C1
Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. H. Prapavessis
Review Number: 16306 **Review Level:** Full Board
Review Date: July 07, 2009
Protocol Title: Getting physical on cigarettes
Department and Institution: Kinesiology, University of Western Ontario
Sponsor: NCIC-NATIONAL CANCER INSTITUTE OF CANADA
Ethics Approval Date: October 01, 2009 **Expiry Date:** March 31, 2013
Documents Reviewed and Approved: UWO Protocol, Letter of information & consent form, advertisement, & recruitment script
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. Joseph Gilbert

Ethics Officer to Contact for Further Information			
☑ Janice Sutherland (jsuther@uwo.ca)	☐ Elizabeth Wambolt (ewambolt@uwo.ca)	☐ Grace Kelly (grace.kelly@uwo.ca)	☐ Denise Grafton (dgrafton@uwo.ca)

This is an official document. Please retain the original in your files.

cc: ORE File

Office of Research Ethics

The University of Western Ontario
Room 4180 Support Services Building, London, ON, Canada N6A 5C1
Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. J.K. Shoemaker
Review Number: 16921 **Review Level:** Full Board
Review Date: February 23, 2010 **Approved Local # of Participants:** 240
Protocol Title: Normative Vascular Indices Across the Lifespan
Department and Institution: Kinesiology, University of Western Ontario
Sponsor: NATIONAL SCIENCE AND ENGINEERING RESEARCH COUNCIL
Ethics Approval Date: March 23, 2010 **Expiry Date:** March 31, 2015
Documents Reviewed and Approved: UWO Protocol, Letter of information & consent form & Assent for Ages 13 to 17 & Advertisements
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. Joseph Gilbert
FDA Ref. #: IRB 00000940

Ethics Officer to Contact for Further Information			
☑ Janice Sutherland (jsutherl@uwo.ca)	☐ Elizabeth Wambolt (ewambolt@uwo.ca)	☐ Grace Kelly (grace.kelly@uwo.ca)	☐ Denise Grafton (dgrafton@uwo.ca)

This is an official document. Please retain the original in your files. cc: ORE File